DISEASES of POULTRY

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INTRODUCTION

Richard K. Gast

Infections with bacteria of the genus *Salmonella* are responsible for a variety of acute and chronic diseases in poultry. Infected poultry, moreover, comprise one of the most important reservoirs of salmonellae that can be transmitted through the food chain to humans. Isolations of *Salmonella* are reported more often from poultry and poultry products than from any other animal species. This likely reflects not only the high prevalence of *Salmonella* infections in poultry, but also the very large numbers of commercially raised chickens and turkeys, and the application of active nationwide programs for identifying infected flocks.

The genus *Salmonella* (of the family Enterobacteriaceae), named for the eminent United States Department of Agriculture (USDA) veterinarian and bacteriologist Daniel E. Salmon, consists of more than 2300 serologically distinguishable variants. These serotypes are usually named for the place of initial isolation. Although recent taxonomic refinements have indicated that all salmonellae can be grouped into only five subgenera (1), the distinctions between serotypes are often epidemiologically relevant. Accordingly, *Salmonella* isolates are still most often described primarily in terms of their traditional serotype nomenclature.

Infections of poultry with salmonellae can be grouped into three categories, each of which is the subject of a separate section of this chapter. The first section discusses infections with the two nonmotile serotypes, *S. pullorum* and *S. gallinarum*, which are generally host-specific for avian species. Pullorum disease, caused by *S. pullorum*, is an acute systemic disease of chicks and poults. Fowl typhoid, caused by *S. gallinarum*, is an acute or chronic septicemic disease that most often affects mature birds. Both of these diseases have been responsible for serious economic losses to poultry producers in the past, and have been addressed by the implementation of extensive testing and eradication programs.

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The second section of this chapter discusses infections with a group of motile *Salmonella* serotypes referred to collectively as paratyphoid salmonellae. This diverse group of serotypes is principally of concern as a cause of food-borne disease in humans. Although paratyphoid infections of poultry are very common, they seldom cause acute systemic disease except in highly susceptible young birds subjected to stressful conditions. More often, paratyphoid *Salmonella* infections of chickens and turkeys are characterized by asymptomatic colonization of the intestinal tract, sometimes persisting until slaughter and leading ultimately to contamination of the finished carcass. Some serotypes, especially *S. enteritidis*, can be deposited in the contents of clean and intact eggs. Improper food handling before consumption can permit the multiplication of *Salmonella* to levels capable of causing severe gastrointestinal disease in human consumers. Heightened concerns about the microbial safety of foods have led to the initiation of numerous testing efforts to detect paratyphoid salmonellae in poultry flocks and poultry products.

The third section of this chapter discusses infections with the various motile serotypes of the subgenus *S. arizonae*, which was formerly designated *Arizona hinshawii*. This group of organisms, although biochemically distinct, causes a disease that is not clinically distinguishable from other *Salmonella* infections. Arizonosis is of particular economic significance in turkeys.

The dimensions of the poultry *Salmonella* problem have expanded considerably in recent years. In the past, the primary motivation for controlling *Salmonella* infections in poultry was to reduce disease losses. Today, public health concerns, political pressures, and consumer demands have increasingly made prevention of food-borne transmission of disease to humans an urgent priority for poultry producers. Pullorum disease and fowl typhoid have been attacked effectively in the United States by a strategy of testing and eradication. The paratyphoid salmonellae, however, are not host specific and are found nearly ubiquitously in domestic animals, wild animals, and humans. In addition, the interna-

PARATYPHOID INFECTIONS

Richard K. Gast

INTRODUCTION. Motile *Salmonella* serotypes other than those in the *S. arizonae* subgenus are often referred to as paratyphoid (PT) salmonellae. These organisms can infect a very wide variety of hosts (including humans), in some instances resulting in relatively asymptomatic intestinal carriage and in other instances producing clinical disease. First reported in avian species a century ago in an outbreak of infectious enteritis in pigeons (221), PT infections continue to cause significant disease losses in young poultry. More recently, PT salmonellae have been the subject of intensified interest as agents of food-borne disease transmission to humans. Commercial poultry constitute one of the largest and most important reservoirs of salmonellae that can be introduced into the human food supply. Contaminated poultry meat and eggs have consistently been among the most frequently implicated sources of human *Salmonella* outbreaks. Controlling PT infections has thus become an important objective for the poultry industry from both the public health and economic perspectives.

Public Health Significance. Although many other pathogens have recently received considerable attention, salmonellae remain among the leading sources of food-borne illness throughout much of the world. For example, nearly 84% of food-borne human illnesses in Scotland between 1980 and 1989 for which a causative agent was established were attributed to salmonellae (239). Between 1973 and 1987, 51% of human food-borne bacterial disease cases in the United States were caused by salmonellae. (23). According to the Centers for Disease Control and Prevention, salmonellosis may affect as many as 1–5 million people each year in the United States; about 20,000 hospitalizations and 500 deaths associated with *Salmonella* are reported annually (255). *Salmonella* outbreaks can have particularly severe consequences in highly vulnerable populations. Of 52 food-borne disease outbreaks in nursing homes in the United States between 1975 and 1987 that had known causes, 52% of the outbreaks and 81% of the deaths were associated with *Salmonella* (195).

Poultry products are consistently identified as important sources of salmonellae that cause illness in humans. More than one-third of food-borne salmonellosis outbreaks in humans in the United States between 1983 and 1987 were associated with poultry meat or eggs (297). The percentage of *Salmonella* outbreaks in England and Wales that were associated with poultry meat rose from less than 13% in 1959–62 to more than 32% in 1984–85 (158). Between 1985 and 1991, 82% of *S. enteritidis* outbreaks in the United States that could be attributed to a specific food vehicle were associated with eggs (218).

Economic Significance. Infections of domestic poultry with salmonellae are expensive both for the poultry industry and for society as a whole. The costs associated with PT infections in poultry fall into two broad categories. The first concerns the expenses associated with human illnesses caused by the consumption of contaminated poultry products. The total combined costs associated with medical care and lost productivity resulting from food-borne *Salmonella* infections of humans in the United States have been estimated at up to $3.5 billion for 1993 (318).

The second category of costs associated with salmonellae in poultry involves various direct expenses producers face as a consequence of *Salmonella* infections in their flocks. During the first few days after hatching, *Salmonella* infections acquired vertically from parents or horizontally in the hatchery can cause significant growth depression or even mortality in young chicks or poults. Although birds quickly become far less susceptible to salmonellae during the 1st wk of life, other diseases or stressful conditions can predispose poultry to severe *Salmonella* infections. Likewise, infection with *Salmonella* can increase the susceptibility of birds to other pathogens. Infections of mature poultry with salmonellae can also be costly to producers in terms of the efforts required to prevent the transmission of infection to progeny or to humans. Control measures such as biosecurity practices, cleaning and disinfecting of facilities, rodent control programs, vaccination, and testing all can significantly increase production costs. Moreover, negative publicity generated by media reports regarding *Salmonella* contamination of particular foods can significantly affect consumer demand for those items and, thereby, ultimately affect the profitability of producers.

INCIDENCE AND DISTRIBUTION. Found in virtually every part of the world, salmonellae infect or are carried by an extremely wide variety of hosts, including wild animals, domestic animals, and humans. Information about the incidence and serotype distribution of salmonellae in domestic animal populations is essential for understanding the relationships within and between the reservoirs of

salmonellae in animals and humans that are ultimately responsible for zoonotic disease transmission.

Incidence of Salmonellae in Poultry and Poultry Products. Advances in poultry production practices, changes in consumer lifestyles and preferences, and heightened nutritional awareness have all combined to make poultry products a leading source of protein for much of the world. The incidence of *Salmonella* infection in poultry flocks and the associated incidence of *Salmonella* contamination of poultry products are thus of considerable public health significance. Although salmonellae have been found in poultry flocks of various species, including both meat-type and egg-type breeds, estimates of the incidence of salmonellae in birds or their environments have varied considerably.

Surveys of meat-type poultry have reported the isolation of salmonellae from the feces of 94% of broiler flocks sampled in the Netherlands (319) and from the environments of 87% of turkey flocks sampled in Canada (167). The actual prevalence of infection within *Salmonella*-positive flocks, however, has often been observed to be relatively low (171, 286). Surveys of egg-type poultry have reported the recovery of salmonellae from the feces of 47% of flocks sampled in the Netherlands (319) and from either feces or eggbelt samples from nearly 53% of flocks sampled in Canada (251). In studies of pooled cecal samples from spent egg-laying flocks in the United States, salmonellae were detected in all of 81 flocks from nine southern states (327) and in 86% of 406 houses from several regions (88).

In surveys of poultry products, salmonellae have been isolated from 57% of chicken carcasses in Portugal (201), 43% of ready-to-cook broiler carcasses obtained from retail stores in Ohio (33), and 29% of frozen broiler carcasses obtained from retail stores in Arkansas (169). Contamination of eggs with salmonellae has also become an important issue in recent years. In a study of more than 1000 unpasteurized liquid egg samples collected at 20 egg-breaking plants throughout the United States, Ebel et al. (89) found salmonellae in 52% of the samples.

Distribution of *Salmonella* Serotypes. Although more than 2300 serotypes of *Salmonella* have been identified, only about 10% of these have been isolated from poultry. Moreover, an even smaller subset of serotypes accounts for the vast majority of poultry *Salmonella* isolates. The distribution of *Salmonella* serotypes from poultry sources varies geographically and changes over time. The degree of relatedness between the poultry and human reservoirs of salmonellae is partly illustrated by similarities in the distribution of serotypes.

Although the frequency of isolation of various *Salmonella* serotypes from poultry changes from year to year, several serotypes are consistently found at a high incidence. Based on data from clinical and environmental isolates submitted to the U.S. Department of Agriculture (USDA) National Veterinary Service Laboratory between July 1990 and June 1993, the most commonly identified serotypes in chickens in the United States were (in descending order of incidence) *S. heidelberg*, *S. enteritidis*, *S. hadar*, *S. montevideo*, *S. kentucky*, and *S. typhimurium* (97, 98, 99). These reports also indicated that the most commonly isolated PT salmonellae in turkeys in the United States during the same period were *S. reading*, *S. heidelberg*, *S. hadar*, *S. agona*, *S. senftenberg*, and *S. saintpaul*. The significance of the poultry *Salmonella* reservoir for public health can be illustrated by considering the serotypes commonly isolated from humans. In 1991, the serotypes most often reported to the Centers for Disease Control and Prevention from human sources in the United States were *S. typhimurium*, *S. enteritidis*, *S. heidelberg*, *S. hadar*, *S. newport*, and *S. agona* (24).

Because of the unique epidemiologic association of *S. enteritidis* with disease transmission via contaminated eggs, the specific prevalence of this one serotype has been a topic of considerable interest in recent years. A Canadian report indicated that environmental samples from 2.7% of 295 layer flocks and 3% of 294 broiler flocks were positive for *S. enteritidis* (250). In two nationwide surveys in the United States, *S. enteritidis* was found in pooled cecal samples from 27% of 406 laying houses tested (88) and from 13% of 1002 pooled samples of unpasteurized liquid egg (89). The increasing public health significance of *S. enteritidis* was shown in a survey of the frequency of reporting of human infections with various *Salmonella* serotypes in 21 nations (261). Only 10% of these nations reported *S. enteritidis* as their most common serotype in 1979, but by 1987 this figure had increased to 43%.

ETIOLOGY

Classification and Nomenclature. The genus *Salmonella* is a member of the bacterial family Enterobacteriaceae and is divided into five biochemically distinct subgenera (184). The various motile and non-host-adapted serotypes of subgenus I are often referred to as PT salmonellae. The degree of genetic relatedness among the salmonellae is so great that some researchers have suggested that the genus actually consists of only a single species (93), but the names of individual serotypes

remain in common usage to facilitate diagnostic classification and epidemiologic analysis.

Morphology and Staining. Salmonellae are straight, non-spore-forming rods, measuring about 0.7–1.5 x 2.0–5.0 μm. Salmonellae are gram-negative, but cells can readily be stained with common dyes such as methylene blue or carbolfuchsin. Paratyphoid salmonellae are usually peritrichously flagellated and motile, although naturally occurring nonmotile mutants are occasionally encountered. Typical *Salmonella* colonies on agar media are about 2 to 4 mm in diameter, round with smooth edges, slightly raised, and glistening.

Growth Requirements. Salmonellae are facultatively anaerobic and can grow well under both aerobic and anaerobic conditions. The optimum temperature to support the growth of salmonellae is 37 C, but some growth is generally observed over a range of about 5 to 45 C. Salmonellae can grow within a pH range of about 4.0 to 9.0, with an optimum pH of about 7.0. The nutritional requirements of salmonellae are relatively simple, and most culture media that supply sources of carbon and nitrogen can support their growth. The viability of *Salmonella* cultures can be maintained for many years in simple media, such as peptone agar (184) or nutrient agar, which have been stab-inoculated, sealed, and held at room temperature.

Biochemical Properties. The biochemical properties characteristic of most PT (subgenus I) *Salmonella* strains are described by Krieg and Holt (184) and Ewing (93). Typical PT salmonellae ferment glucose (to produce both acid and gas), dulcitol, mannitol, maltose, and mucate, but do not ferment lactose, sucrose, malonate, or salicin. They can produce hydrogen sulfide on many types of media, decarboxylate ornithine and lysine, utilize citrate as a sole source of carbon, and reduce nitrates to nitrites. Paratyphoid salmonellae do not hydrolyze urea or gelatin and do not produce indole.

Paratyphoid salmonellae can be distinguished from *S. arizonae* (*Salmonella* subgenus III), *S. pullorum*, and *S. gallinarum* on the basis of several biochemical differences. For example, *S. arizonae* strains cannot ferment dulcitol but usually can ferment malonate, *S. pullorum* strains cannot ferment mucate or dulcitol, and *S. gallinarum* strains cannot decarboxylate ornithine or produce gas from glucose fermentation. In addition, PT salmonellae are usually motile, but *S. pullorum* and *S. gallinarum* are nonmotile.

Antigenic Structure. The traditional Kauffmann-White schema for antigenic classification of salmonellae is based on both somatic and flagellar antigens (93). The somatic "O" antigens are determined by polysaccharides associated with the body of the cell and are identified by arabic numerals. Serogroups (designated with upper-case letters) of salmonellae are defined by particular somatic antigens that are unique to members of the group. Most *Salmonella* isolates found in poultry belong to Serogroups B, C, or D. The "H" antigens are determined by flagellar proteins and are usually identified by lower-case letters. Flagellar antigens sometimes occur in two different phases. The serotype of a particular *Salmonella* isolate is determined by the combination of O and H antigens that it expresses. Serotyping of isolates is generally accomplished using agglutination tests with batteries of specific antisera. Slide agglutination tests are first used to establish the somatic antigen content and the flagellar antigen content is then determined using tube agglutination tests.

Resistance to Physical and Chemical Agents

HEAT, IRRADIATION, AND OTHER PHYSICAL AGENTS. With the exception of a few distinctively thermoresistant strains (such as *S. senftenberg* 775W), salmonellae are generally quite susceptible to destruction by heat. For example, cooking to an internal temperature of 79 C in conventional or convection ovens always eliminated inoculated *S. typhimurium* from roasting chickens (269). Exposure of ground chicken meat to a temperature of 60 C eliminated *S. typhimurium* contamination (at a level of 10^4 cells/g) within 5 minutes (22). The heat resistance of *S. enteritidis* can be increased by prior exposure to alkaline conditions (163), and decreased by prior refrigeration (156, 263). Salmonellae strains of several serotypes have been able to survive cooking methods for eggs that allow some of the yolk to remain liquid (12, 161). Liquid whole egg is pasteurized in the United States according to USDA specifications that require a minimum treatment time of 3.5 minutes at 60 C (11). Steam pelleting treatment of poultry feed under precisely defined conditions has been reported to eliminate both inoculated and naturally occurring salmonellae (213, 270).

Irradiation has received considerable attention as a potential method for eliminating salmonellae from foods and feedstuffs. Most salmonellae strains appear to be highly susceptible to the lethal effects of irradiation (302). Gamma radiation has been successfully applied to reducing the levels of *Salmonella* contamination in poultry meat (301, 304), egg products (211, 265), and poultry feeds (193). Combined heat and radiation treatments have been shown to be more effective in eliminating salmonellae than either treatment alone (265, 303). Sev-

eral other physical agents, including electrical stimulation (196, 275), ultraviolet radiation (325), and ultrasonic wave treatment (346) have also been reported to be lethal for salmonellae.

CHEMICAL DISINFECTANTS. A wide variety of chemical disinfectants have been assessed for their efficacy against salmonellae. The application of hydrogen peroxide (226), acetic acid (84), lactic acid (168), potassium sorbate (223), chlorine (223), or trisodium phosphate (175) have all been reported to reduce the incidence or level of *Salmonella* contamination on broiler carcasses. Fumigating with formaldehyde (335) or hydrogen peroxide (272), or spraying with polyhexamethylene biguanide hydrochloride (73), has been shown to be effective in controlling salmonellae on hatching eggs. Both ozone and formaldehyde fumigation have similarly been reported to be effective poultry hatchery disinfectants (332).

Studies of the efficacy of chemical treatment of poultry feeds to inhibit salmonellae have produced variable results. Inclusion of an organic acid mixture in feed was reported to reduce significantly the eventual level of salmonellae in feed contaminated with mouse droppings containing *S. typhimurium* (189). Smyser and Snoeyenbos (277), however, studied 12 compounds as potential antagonists of salmonellae in poultry feed (including organic acids) and found that only formalin was consistently effective.

The application of chemical disinfectants to poultry houses is also of uncertain effectiveness. Phenols and quaternary ammonium compounds are often used for this purpose, but cleaning and disinfection has not always been successful in eliminating salmonellae from contaminated houses (209, 282). Formaldehyde fumigation has been found to be highly effective for this purpose (337), but safety considerations have limited its availability and use.

ENVIRONMENTAL FACTORS. The environmental persistence of PT salmonellae is a significant factor in the epidemiology of these organisms in poultry by creating opportunities for horizontal transmission of infection within and between flocks. Smyser et al. (278) reported the isolation of *S. heidelberg* from contaminated litter after 7 mo of holding at room temperature. Williams and Benson (338) observed the survival of *S. typhimurium* for 16 mo in feed and 18 mo in litter stored at 25 C. Water activity has been identified as an important supporting factor in allowing the persistence of salmonellae in poultry houses (242). Although salmonellae can sometimes persist for long periods in poultry litter, used litter has also occasionally been reported to exert an inhibitory effect on *Salmonella* growth or survival (312). Turnbull and Snoeyenbos (313) suggested that this effect might result from a pH increase over time due to dissolved ammonia. The survival of salmonellae on eggs during and after washing has been shown to be dependent on the pH, temperature, and presence of egg solids in the washwater (190) and on the rate of cooling after washing (42).

Pathogenicity

TOXINS. Three general categories of toxins have been reported to play roles in the pathogenicity of PT salmonellae. Endotoxin is associated with the lipid A portion of *Salmonella* cell wall lipopolysaccharide (LPS). If released into the bloodstream of an infected animal when bacterial cells are lysed, endotoxin can produce fever. Intravenously administered *S. enteritidis* endotoxin was found by Turnbull and Snoeyenbos (314) to cause liver and spleen lesions in 2-wk-old chickens. Lipopolysaccharide also contributes to the resistance of the bacterial cell wall to attack and digestion by host phagocytes. Loss of the ability to synthesize complete LPS has been associated with a loss of virulence for *S. enteritidis* in mice (45) and an impaired ability of *S. typhimurium* to colonize the ceca and invade to the spleen in broiler chicks (74).

Two proteinaceous toxins have also been identified in salmonellae. Enterotoxin activity by salmonellae induces a secretory response by epithelial cells that results in fluid accumulation in the intestinal lumen (183). A heat-labile enterotoxin was detected in 44% of 123 *S. typhimurium* strains from animal sources (214). The heat-stable cytotoxin of salmonellae causes structural damage to intestinal epithelial cells, perhaps by inhibiting protein synthesis (181).

Adherence, Invasiveness, and Intracellular Survival. The adherence of PT salmonellae to intestinal epithelial cells is the pivotal first step in the sequence of events that produces disease. Adherence has been associated with type 1 fimbriae (5, 198) and with a mannose-resistant hemagglutinin (101). Although adherence evidently does not require metabolically active salmonellae, the subsequent bacterial invasion of host cells requires protein synthesis by live salmonellae (186, 200). The overall virulence of salmonellae depends heavily on the initial degree of mucosal invasiveness (4). Putative mechanisms for *Salmonella* invasion of intestinal cells have included type 1 fimbriae (92) and various bacterial proteins induced by contact with epithelial cell surfaces (102).

Adherence and invasiveness of salmonellae can be influenced by culture growth conditions. Logarithmically growing *Salmonella* cells are more invasive in tissue culture than are cells in the station-

ary phase of growth, and salmonellae grown anaerobically have been shown to be both more adherent and more invasive than salmonellae grown aerobically (92, 191). Rapidly growing *S. typhimurium* cells have been reported to kill mice faster after intravenous injection than do cells in a slower phase of growth (27).

The replication of salmonellae within host cells has also been found to be necessary for the full expression of pathogenicity (194). Mutants of *S. typhimurium* that were unable to survive within host macrophages (100) or to resist the antimicrobial effects of host peptides (127) were reported to exhibit reduced virulence in mice. The production of iron-chelating siderophores may also contribute to the in vivo survival of salmonellae (349).

PLASMIDS. Plasmids are extrachromosomal DNA elements that have often been associated with bacterial pathogenicity. Serotype-specific plasmids of characteristic molecular weights have been directly linked with virulence for a number of salmonellae. Considerable homology has been demonstrated between virulence-associated plasmids of different serotypes (36, 344, 345). Strains of *S. typhimurium* and *S. enteritidis* cured of their virulence-associated plasmids have been found to be significantly less lethal for mice (46, 49, 137, 228). Plasmid-mediated virulence among *S. typhimurium* and *S. enteritidis* isolates has been variously associated with invasion of mesenteric lymph nodes, the liver, and the spleen in mice (128, 293), in vivo growth within cells of infected mice (129, 154, 260), immunosuppression (144), and serum resistance (292). Analysis and characterization of the plasmid content of *S. enteritidis* isolates from diverse poultry sources has proven to be of significant value in establishing epidemiologic relationships (85, 274).

The pathogenicity of salmonellae, however, does not always require the presence of the serotype-specific plasmids. Some strains of *S. typhimurium*, for example, have been shown to retain their invasiveness in cell culture assays (37, 153) and their lethality for infected mice (244) in the absence of virulence-associated plasmids. Moreover, although a serotype-specific plasmid was found to be essential for the full expression of virulence by *S enteritidis* in mice, curing this plasmid did not affect *S. enteritidis* colonization and invasion of the tissues of orally inoculated chickens (130).

Pathogenicity Differences of Strains, Serotypes, and Phage Types. Paratyphoid salmonellae strains are often found to differ in their ability to cause disease or death in young poultry. Several investigators (58, 138, 276) have reported significant differences in mortality between groups of chicks orally inoculated with isolates representing diverse *Salmonella* serotypes. However, tremendous variation in lethality for chicks has also been observed within single *Salmonella* serotypes, sometimes even among strains of the same phage type (16, 276). Pathogenicity differences have also been noted between the various phage types of *S. enteritidis*, with phage type 4 often associated with a particularly high level of invasiveness (13, 143) and lethality (13, 114, 253) for newly hatched chicks. Differences in virulence within the phage types of *S. enteritidis* (including phage type 4), however, have also been demonstrated (112, 114, 253). Variation between strains of *S. enteritidis*, crossing phage-type boundaries, has also been reported in the frequency of deposition in the contents of eggs laid by experimentally infected hens (112, 273).

The bacterial characteristics responsible for the observed pathogenicity differences between *Salmonella* strains are still incompletely understood. Nolan et al. (237) compared *Salmonella* isolates of identical serotypes obtained from healthy and ill chickens and found differences in the utilization of carbon sources, mannose-sensitive hemagglutination of erythrocytes, and invasiveness in cell culture. However, Barrow et al. (17) concluded that flagellar and somatic antigens, mannose-sensitive hemagglutinins, and the serotype-specific plasmid of *S. typhimurium* were all unessential for intestinal colonization. Petter (246) associated invasive properties of *S. enteritidis* variants with quantitative and qualitative differences in LPS expression.

PATHOGENESIS AND EPIZOOTIOLOGY.

Paratyphoid salmonellae can be isolated from an extremely wide variety of host species, including humans and other mammals, birds, reptiles, and insects. The many interconnections between these reservoirs often impair efforts to reduce the incidence of *Salmonella* infections in humans and domestic animals. Poultry have often been identified as one of the most important reservoirs of salmonellae that ultimately cause human infections. Although chickens and turkeys are susceptible to a broad range of *Salmonella* serotypes, the resulting infection process is determined less by the serotype involved than by factors such as the age of the affected birds, the infecting dose, and predisposing conditions. Paratyphoid salmonellae can be introduced into poultry flocks by several different sources and can likewise be spread within and between flocks by a number of mechanisms.

Paratyphoid Infections in Young Poultry. Paratyphoid infections often have very different consequences for newly hatched poultry than for more mature birds. In very susceptible young chicks and poults, PT infection can sometimes lead

to illness and death at high frequencies. Older birds are far less susceptible to the lethal effects of PT salmonellae and may experience intestinal colonization and even systemic dissemination without significant morbidity or mortality. The development of resistance to salmonellae in young birds has often been attributed to the acquisition of protective microflora that either compete with salmonellae for intestinal receptor sites or produce antagonistic factors that inhibit *Salmonella* growth (285, 290). Gast and Beard (107) accordingly observed that significantly more orally administered *S. typhimurium* cells adhered in the ceca of 2-day-old chicks than in those of 3- to- 7-day-old chicks.

The usual outcomes of PT infections in chicks and poults fall into three general categories (271). Intestinal colonization is normally the first step in the infection process for orally introduced PT salmonellae, frequently leading to the persistent shedding of salmonellae in the feces. In many infected birds, invasion beyond the gastrointestinal tract results in *Salmonella* multiplication in reticuloendothelial tissue of the liver and spleen (16) and eventual dissemination to colonize a variety of internal tissue sites. Finally, extensive bacteremia sometimes occurs, occasionally causing a high incidence of mortality. The incidence of both mortality (95) and intestinal colonization (262) in chicks correlate strongly with the dose of orally administered salmonellae.

Mortality associated with naturally occurring PT infections in poultry is often observed to reach peak levels at about 3 to 7 days of age (222). Studies of experimental PT infections in young poultry have consistently shown that newly hatched birds are highly susceptible to salmonellae, but this susceptibility decreases over time. Fagerberg et al. (95) found that oral doses of 10^9 *S. typhimurium* cells were lethal for 50% of 1-day-old broiler chicks, 20% of 3-day-old chicks, and no 7-day-old chicks. Smith and Tucker (276) saw mortality associated with *S. typhimurium* inoculation of chicks drop precipitously from 79% at 1 day of age to only 3% at 2 days. A steep reduction in susceptibility to PT-associated mortality has also been reported in turkey poults (31).

The frequency of both intestinal colonization (262) and invasion to internal organs (314) are higher in newly hatched chicks than in older birds. The persistence of salmonellae in various colonization sites is also influenced by the age of the birds when infected. Horizontal contact exposure of chicks within 24 hr of hatching has been reported to result in fecal shedding of *S. enteritidis* for at least 28 wk (230). Gast and Beard (107) determined that cecal colonization with *S. typhimurium* persisted for 7 wk after oral inoculation significantly more often when chicks were infected at 1 day of age than at 7 days. Gorham et al. (125) likewise observed an age-related decrease in the persistence of *S. enteritidis* in the internal organs of inoculated chicks.

Paratyphoid Infections in Mature Poultry. Morbidity or mortality are not consistently associated with PT infections in mature poultry. Experimental infections of adult chickens with large oral doses of PT salmonellae have often been reported to cause no evident signs of clinical illness (35, 159). Timoney et al. (309) noted that although oral inoculation of laying hens with *S. enteritidis* often resulted in bacteremia and extensive systemic dissemination to internal organ sites, the birds remained clinically normal except for some brief mild diarrhea. Humphrey et al. (162) observed, however, that six of ten 1-year-old hens died after oral inoculation with a phage type 4 *S. enteritidis* isolate.

The two most consistently observed features of PT infections in mature poultry are intestinal colonization and systemic dissemination to internal organs. During approximately the first 2 wk following experimental oral infection of chickens or turkeys, PT salmonellae can generally be isolated from the intestinal tracts and voided feces of a high percentage of inoculated birds (35, 110, 348). Although the incidence of intestinal colonization and fecal shedding steadily declines thereafter, some *S. enteritidis* strains have been shown to persist in the intestinal tract of laying chickens for several months after oral inoculation (108, 110, 273).

Gut colonization by PT salmonellae is usually followed by invasion of the intestinal epithelium and dissemination to internal tissues. Various PT serotypes, including *S. infantis*, *S. typhimurium*, and *S. heidelberg*, have been found in internal sites such as the liver, spleen, lung, ovary, oviduct, and peritoneum of naturally and experimentally infected chickens and turkeys (35, 281, 348). Invasiveness and systemic dissemination have been documented very extensively for *S. enteritidis*. After experimental oral inoculation of laying hens, *S. enteritidis* has been isolated from numerous internal tissues, including the liver, spleen, ovary, oviduct, heart blood, and peritoneum (110, 309). Dissemination of *S. enteritidis* to diverse internal organs, including the ovary and oviduct, has also been recorded following conjunctival inoculation (165) or exposure to contaminated aerosols (21). The isolation of *S. enteritidis* from a wide range of internal organs has similarly been reported in naturally infected poultry (152, 151, 252).

Another aspect of infections of mature chickens with some PT salmonellae that is of particular concern from a public health perspective is the production of *Salmonella*-contaminated eggs. In the late 1980s, considerable epidemiologic evidence began

to accumulate indicating that the contaminated contents of clean and intact eggs were responsible for the transmission of *S. enteritidis* infection to humans (217, 287). Investigations of laying flocks implicated as the sources of eggs that caused human outbreaks have detected *S. enteritidis* isolates of the same phage types found in affected humans, often with identical plasmid profiles or fingerprints, in environmental samples, tissue samples, and eggs (86, 140, 306).

S. enteritidis has been found in the contents of eggs laid by commercial layers (152) and broiler breeders (199), but the incidence of *S. enteritidis* contamination of eggs has generally been found to be extremely low. In studies of 17 naturally infected laying flocks in the United Kingdom, Humphrey et al. (160, 164) found *S. enteritidis* in the contents of less than 1% of the eggs sampled. In two Canadian layer flocks that yielded *S. enteritidis* isolates from both environmental and tissue samples, less than 0.06% of the eggs sampled were contaminated with *S. enteritidis* (252). Naturally contaminated eggs have generally been found to contain very small numbers of *S. enteritidis* (160, 164), but the *S. enteritidis* population in eggs can expand to more dangerous levels if eggs are held at growth-supporting temperatures (113, 164). Contamination of egg contents by *S. enteritidis* has also been demonstrated in experimentally infected laying hens (273, 309). Gast and Beard (108) isolated *S. enteritidis* from the albumen of 19% and yolks of 16% of eggs laid within 4 wk after the administration of a large oral dose to hens.

Predisposing Factors. A number of factors have been demonstrated to increase the likelihood or severity of PT infection in poultry. Several other infectious agents have been reported to influence the course of infection with salmonellae. Prior infection with several species of coccidia, including *Eimeria tenella, E. maxima,* and *E. acervulina,* can increase the ability of salmonella serotypes such as *S. typhimurium, S. enteritidis, S. agona,* and *S. infantis* to colonize the intestinal tracts of chickens (8, 256, 294). The mechanisms for this effect may be related to decreased levels of *Salmonella*-inhibiting volatile fatty acids and increased oxidation-reduction potential in the intestine related to coccidial infection (7). Infection with *E. tenella,* however, was observed by Tellez et al. (299) to decrease the frequency by invasion of subsequently administered *S. enteritidis* to the internal organs of chicks, perhaps by increasing the thickness of the intestinal lamina propria. Infections of poultry with immunosuppressive viruses or bacteria can also affect the outcome of *Salmonella* infections. Exposure to reticuloendotheliosis virus at 1 day of age increased mortality among chicks inoculated intraperitoneally with *S. typhimurium* at 1, 7, or 14 days of age (224). Exposure of 1-day-old chicks to infectious bursal disease virus led to increased mortality following *S. typhimurium* infection 3 wk later, although viral infection of 3-wk-old chicks did not affect their susceptibility to subsequent infection with *S. typhimurium* (347). Suppression of cell-mediated immunity by *Corynebacterium parvum* led to increased morbidity in chicks subsequently infected with *S. typhimurium* (60).

Environmental and management factors can also influence the susceptibility of poultry to PT salmonellae. Exposure to stressful conditions has often been shown to facilitate or exacerbate *Salmonella* infections. For example, lowering the brooding temperature of chicks by 5 to 8 C was found to increase significantly mortality among newly hatched chicks inoculated with *S. worthington* (300). Water deprivation before inoculation of 7-wk-old chickens increased the duration of fecal shedding of orally administered *S. typhimurium* (34). Forced molting of laying hens by feed deprivation has been reported to increase the incidence and level of fecal shedding (149), the incidence and severity of intestinal lesions (147, 254), and the frequency of horizontal transmission (146) following oral inoculation with *S. enteritidis.* Molting also reduced the infectious dose of *S. enteritidis* necessary to establish intestinal colonization in hens (145) and increased the likelihood of recurrence of previous *S. enteritidis* infections (148).

Sources, Vectors, and Transmission. Paratyphoid salmonellae can be introduced into poultry flocks from many different sources. The extremely wide host range of PT salmonellae creates an equally large number of reservoirs of infectious organisms that can be transmitted to chickens or turkeys. Among the most frequently implicated sources of infection are contaminated feed and various animal and insect vectors. Paratyphoid salmonellae can be transmitted vertically to the progeny of infected breeder flocks and horizontally within and between flocks.

Contaminated feeds, particularly those containing animal proteins, have often been identified as likely sources of introduction of PT salmonellae to poultry flocks. Zecha et al. (350) reported that four of eight *Salmonella* serotypes isolated from a turkey breeding facility over a 5-yr period had also been isolated from samples of pelleted feed. MacKenzie and Bains (202) noted that *Salmonella* serotypes not previously detected in the flocks of a broiler company in Australia were detected first in raw feed ingredients, and then later appeared in live birds and processed carcasses. Cox et al. (70) collected poultry feed from commercial mills in the United States and found salmonellae in 92% of

meat and bone meal samples and in 58% of finished feed (mash) samples, but in no samples of pelleted feed. Experimental inoculation studies have demonstrated that chicks can readily become infected with PT salmonellae from their feed, even when contamination levels are very low (142, 266).

Biologic vectors can both disseminate and amplify salmonellae in poultry flocks. Insects, including cockroaches (182) and lesser mealworms (212), can carry *Salmonella* organisms internally and externally and spread them throughout poultry houses. Mice have been identified as particularly important vectors for *S. enteritidis* in laying flocks. Henzler and Opitz (139) cultured mice and environmental samples from laying farms to isolate *S. enteritidis*. They detected *S. enteritidis* in 24% of the mice from environmentally contaminated laying farms, but in none of the mice from farms with environments free of *S. enteritidis*. They noted that a single mouse fecal pellet could contain 10^5 *S. enteritidis* cells.

Vertical transmission of PT salmonellae to the progeny of infected breeder flocks can result from the production of eggs contaminated by salmonellae in the contents or on the surface. Experimentally infected hens were observed by Gordon and Tucker (123) to transmit *S. menston* to their offspring. The same *Salmonella* serotypes responsible for mortality in naturally infected chicks and poults have often also been isolated from their parent flocks (185, 222). In a survey of 10 farms in France, Lahellec et al. (188) concluded that the greatest contribution to the eventual distribution of *Salmonella* serotypes in broiler houses came from the chicks themselves and not from their environment.

Egg shells are often contaminated with PT salmonellae by fecal contamination during oviposition. The penetration of salmonellae into or through the shell and shell membranes can result in direct transmission of infection to the developing embryo or can lead to exposure of the chick to infectious *Salmonella* organisms when the shell structure is disrupted during hatching. Some PT serotypes, particularly *S. enteritidis*, can be deposited in the contents of eggs before oviposition. The resulting transovarian transmission of infection to progeny is an important aspect of the epidemiology of *S. enteritidis* in chickens.

Regardless of the mechanism or site of egg contamination, any PT salmonellae carried in or on eggs can be spread extensively in the hatchery. As chicks or poults pip through egg shells, salmonellae are released into the air and circulated around hatching cabinets on contaminated fluff and other hatching debris. Bailey et al. (10) reported that 17% of egg shell samples and 21% of chick rinse samples obtained from commercial broiler hatcheries in the United States were positive for PT salmonellae. Cox et al. (71) likewise isolated salmonellae (of 12 different serotypes) from more than 75% of samples of egg fragments, belting material, and paper pads from three broiler hatcheries. Newly hatched birds, lacking protective intestinal microflora, are highly susceptible to intestinal colonization by salmonellae. Cason et al. (41) observed that nearly 44% of chicks from uncontaminated eggs, hatched along with eggs dipped before incubation in a solution containing *S. typhimurium*, were found to carry *S. typhimurium* in their intestinal tracts upon removal from the hatcher. Bhatia and McNabb (30) found the same *Salmonella* serotypes in hatchery fluff and meconium as were later detected in broiler house litter and finished broiler carcasses.

After introduction into poultry, PT salmonellae can spread horizontally within and between flocks. Snoeyenbos et al. (280) noted that 10 *Salmonella* serotypes spread rapidly from infected day-old chicks to penmates reared on litter. Gast and Beard (108, 110) reported that *S. enteritidis* could be found in the feces and internal organs of uninoculated laying hens housed in cages adjacent to those of orally inoculated birds. Contaminated poultry house environments are often implicated as among the principal sources of PT salmonellae (185). Lahellec and Colin (187) concluded that *Salmonella* serotypes present in broiler houses or introduced into houses by vectors during the rearing period were more likely to appear on processed carcasses than were serotypes originating in the hatchery. In a Dutch study, cumulative infection curves for laying flocks showed an increasing incidence of *S. enteritidis* over time during the laying cycle, suggesting that infection was more likely acquired from farm environments than from breeding stock (321). Horizontal transmission can be mediated by direct bird-to-bird contact, ingestion of contaminated feces or litter, contaminated water (123, 231), personnel and equipment (350), and a variety of other mechanisms.

Clinical Signs. Paratyphoid infection of poultry is usually associated with disease only in very young birds. The contamination of eggs with salmonellae may lead to a high level of embryo mortality and the rapid death of newly hatched birds before clinical signs are observed. Signs of disease are rarely observed after the first 2 wk of life, although morbidity and mortality can be high during that period and significant growth retardation can occur. The course of illness is normally relatively brief in individual birds. Signs of severe PT infection in young poultry are generally similar to those observed in connection with other avian *Salmonella* infections (pullorum disease, fowl typhoid, and avian arizonosis) and with other bacteria that can cause acute septicemia. Although clinical disease is

not normally associated with PT infections in mature poultry, some *S. enteritidis* strains have been found to cause anorexia, diarrhea, and reduced egg production in experimentally infected laying hens (108, 112, 229, 273).

Typical signs of PT infection in chicks and poults include progressive somnolence with closed eyes, drooping wings, and ruffled feathers (336). Anorexia and emaciation are common (16). Affected birds are often seen to shiver and huddle near heat sources. Profuse watery diarrhea is frequently observed, often resulting in dehydration and pasting of the vent area (219, 336). Blindness (245) and lameness (245, 336) have occasionally been associated with PT infections.

Gross Lesions and Histopathology. In severe outbreaks of PT infection in newly hatched poultry, rapidly developing septicemia can cause a high incidence of mortality with few or no apparent lesions. When the course of disease is longer, severe enteritis is often accompanied by focal necrotic lesions in the mucosa of the small intestine. Cheesy cecal cores (16, 126, 336) are often observed. Spleens and livers are commonly swollen and congested, with evident hemorrhagic streaks or necrotic foci (245, 336). Kidneys may also sometimes be enlarged and congested (219). Fibrinopurulent perihepatitis and pericarditis have been reported on numerous occasions (13, 126, 245, 336). Unabsorbed, coagulated yolk material may be present in the yolk sac (13, 126, 245). Other lesions occasionally observed include hypopyon, panophthalmitis, purulent arthritis (245), airsacculitis, (126), and omphalitis (28).

The invasion of intestinal epithelial cells by salmonellae leads to a series of pathologic changes that affects intestinal fluid and electrolyte regulation. This process can ultimately cause cell death and thereby produce and exacerbate diarrhea. Oral inoculation of laying hens with *S. enteritidis* can produce inflammation of the epithelium and lamina propria of the colon and ceca related to heterophilic infiltration (147, 254). Epithelial cells can be invaded throughout the intestinal tract, but the ceca and the ileocecal junction are often sites of particular affinity for salmonellae (314). In addition, epithelial invasion may also allow the removal of salmonellae through the basement membrane into the lamina propria by macrophages (249). Humphrey et al. (166) recovered *S. enteritidis* from several internal organ sites of a few laying hens within as little as 1 hr after oral inoculation. The ability of salmonellae to survive and multiply in internal organs, particularly the liver and spleen, has been correlated with the comparative virulence of salmonellae in different host species (20). Intracellular replication in the spleens of mice has been shown to offer a protected site where bacterial multiplication can continue without exposure to host defense mechanisms (87). Slight inflammatory processes with heterophil infiltration ranging from focal to diffuse in distribution have been observed in the ovaries and oviducts of flocks naturally infected with *S. enteritidis* (151).

Immunity. The immune response of poultry to PT salmonellae acts to minimize the duration and severity of infection and helps protect against reinfection. This response also permits the serologic detection of infected flocks and serves as the basis for efforts to protect birds against infection by vaccination. The development of immunity was illustrated in a study conducted by Hassan et al. (135), in which oral reinfection of chickens with *S. typhimurium* (10 wk after the initial inoculation) resulted in reduced fecal shedding and more rapid clearance from tissues than was observed in previously uninfected birds. Administering immunosuppressive agents to chicks has been reported to increase mortality associated with PT infection (91, 351), but such treatments apparently have very little effect on intestinal colonization by salmonellae (61). Hassan and Curtiss (132) have recently provided evidence that *S. typhimurium* infection of chickens can cause lymphocyte depletion, atrophy of lymphoid organs, and immunosuppression that may facilitate the establishment of a persistent carrier state.

Paratyphoid salmonellae can elicit strong antibody responses from infected poultry. For example, experimental infection of chicks with *S. typhimurium* induced strong IgG, IgA, and IgM responses in serum, intestinal contents, and bile which could be detected by antigens composed of whole bacterial cells, LPS, flagella, and outer-membrane proteins (135). When laying hens were orally infected with *S. enteritidis*, serum antibodies were produced by most birds by 1 wk postinoculation and reached peak values at 1 wk postinoculation (109). High serum IgG titers have been detected in laying hens for at least 27 wk after experimental oral inoculation with *S. enteritidis* (15). In a naturally infected broiler breeder flock, 70% of the birds were found to be positive for serum antibodies to *S. enteritidis* LPS at 35 wk of age (59). Antibodies to *S. enteritidis* have also been found in the yolks of eggs laid by infected hens. Specific antibodies were found as early as 9 days postinoculation and reached peak levels at 3–5 wk postinoculation in eggs from hens experimentally infected with *S. enteritidis* (111). Antibodies to *S. enteritidis* have also been detected in eggs from naturally infected flocks (59).

Although less completely characterized than the antibody response, cell-mediated immunity to PT

salmonellae has also been observed in poultry. Hassan et al. (135) detected a strong delayed hypersensitivity reaction, using either whole bacterial cells or outer membrane proteins, between 2 and 5 wk after experimental infection of chicks with *S. typhimurium*. Heterophils of chickens and turkeys are strongly phagocytic and bactericidal for salmonellae (288) and apparently play a vital role in restricting organ invasion during the early phases of *S. enteritidis* infection (179). Cytokines produced by sensitized T lymphocytes may play a particularly important role in conferring immunity on poultry, perhaps by expanding the pool of circulating phagocytic heterophils (178) and recruiting them to the site of infection (180). Prophylactic administration of these immune lymphokines to chicks has been shown to provide protection against organ invasion by *S. enteritidis* (298).

The relative contributions of the antibody response and the cell-mediated response in providing poultry with protective immunity against *Salmonella* infection are somewhat uncertain. Lee et al. (192) indicated that the development of high antibody levels in chickens experimentally infected with *S. typhimurium* did not seem to result in any significant reductions in the *Salmonella* levels in various tissue sites, but effective clearance of salmonellae from tissues was observed after the emergence of a strong cell-mediated response. On the other hand, Humphrey et al. (162) noted that a group of hens infected with *S. enteritidis* at 20 wk of age produced high levels of IgM antibodies and showed no adverse signs, whereas a group of hens infected at 1 year of age produced much lower levels of antibodies and accordingly experienced significant mortality. Research in mice has indicated that the opsonic activity of specific antibodies and the phagocytic and lytic activity of cellular effectors may both be necessary for the full expression of immunity (210).

Although the responsible mechanism has not been clearly defined, genetically based differences in the innate resistance of lines of chickens to *Salmonella* infection have been reported on several occasions. Chicks from different lines have been found to vary in their susceptibility to the lethal effects of *S. typhimurium* and *S. enteritidis* infection (25, 38). Differences in the incidences of fecal shedding, organ invasion, and egg contamination have been reported between lines of mature chickens infected with *S. enteritidis* (25, 197). Bumstead and Barrow (39) found that the patterns of susceptibility of six inbred lines of chickens to various host-adapted and PT *Salmonella* serotypes were all very similar, suggesting a common mechanism of resistance.

DIAGNOSIS. Although clinical observations may suggest the likelihood of a PT infection, final diagnosis depends on the isolation and identification of causative organisms. Using conventional culture methods, this requires 48 to 96 hr (and even longer for some culturing protocols). A concise summary of traditional methods for isolating salmonellae from poultry was provided by Mallinson and Snoeyenbos (205). A wide array of faster alternative strategies for detecting and identifying salmonellae have also been proposed in recent years. Serologic detection of specific antibodies is often employed effectively as a rapid preliminary screening device to identify flocks that have been exposed to salmonellae.

Isolation and Identification of Causative Agent

SAMPLE SELECTION. To identify PT infection in poultry flocks, samples are obtained and cultured from a variety of sources, principally including tissues, eggs, and the poultry house environment. The number of samples that must be processed to achieve a predetermined level of confidence of detection of PT infection in a flock is directly related to the size of the flock and inversely related to the actual prevalence of infection (1). In very large flocks estimated to have very low prevalences of *Salmonella* infection, samples from more than one bird are often pooled together before culturing to allow an adequate sample size to be attained within the limitations of existing laboratory resources.

As many PT *Salmonella* serotypes are highly invasive and can be systemically disseminated to numerous internal tissues, a diversity of different sites (including the liver, spleen, ovary, oviduct, testes, yolk sac, heart, heart blood, kidney, gall bladder, pancreas, synovia, and eye) can provide samples for diagnostic culturing. As lesions cannot be relied upon to indicate infected tissues, several different organs should be cultured from each bird (separately or together). Some highly invasive PT serotypes, particularly *S. enteritidis*, can be deposited in the contents of eggs before oviposition (108). Culturing eggs for *S. enteritidis*, therefore, has been applied as a test for assessing the potential threat to public health posed by infected laying flocks. Gast (103) reported that culturing pools of egg contents for *S. enteritidis* detected experimentally infected hens at a frequency similar to culturing fecal samples or testing for specific serum antibodies during the first 2 wk after inoculation.

Because infections of poultry with PT salmonellae almost invariably involve colonization of the intestinal tract, samples of intestinal tissues and contents are frequently the focus of *Salmonella*-culturing efforts. In a survey of birds submitted to a diagnostic laboratory (94), salmonellae were found exclusively in intestinal samples in 78% of the chickens and 70% of the turkeys. In experimentally

inoculated laying hens, *S. enteritidis* was recovered more often from the intestinal tract than from any other tissue sampled (110). Most recommendations for culturing intestinal samples indicate that the caudal ileum, ceca, cecal tonsils, and cecal contents are the sites most likely to offer the maximum probability of recovering salmonellae (34, 96, 314). Cloacal swabs (108) or samples of voided feces (103) have been used to provide evidence of persistent intestinal colonization by salmonellae in individual birds. The often intermittent pattern of shedding of salmonellae in the feces of infected birds tends to diminish the overall reliability of cloacal swabs for diagnosing infection (203, 341).

Fecal shedding of salmonellae into the poultry house environment by infected birds makes culturing environmental samples a useful diagnostic tool. Moreover, environmental samples also provide an opportunity to monitor for the introduction of salmonellae into poultry houses by vectors, personnel, equipment, and other sources. Although sampling fresh feces themselves likely provides the most sensitive test for the shedding of salmonellae (141), sampling litter can sometimes provide a comparable level of detection (264). Olesiuk et al. (240) reported that experimental *S. typhimurium* infection in laying flocks was detected more consistently over a period of 1 yr by culturing floor litter than by any other testing approach. In a naturally infected laying flock, Snoeyenbos et al. (279) found that salmonellae were most often recovered from nest litter samples. Drag-swab samples, obtained by dragging moistened gauze pads across the floor of poultry houses, have been reported to detect salmonellae with greater sensitivity than litter sampling (176). The use of multiple-swab assemblies can further improve the sensitivity of this method (40).

Numerous other environmental sampling approaches, including the culturing of cage surfaces, water sources, eggbelts, trapped rodents, and dust have also been suggested. Dust can remain contaminated with salmonellae even after cleaning and disinfection of poultry houses (141). Hatcher fluff is frequently contaminated with salmonellae, offering an opportunity for early detection of infection in flocks (220, 264). Culturing poultry feed for salmonellae is often important in establishing the source of infection of a flock with a particular serotype (279).

STANDARD CULTURE METHODS FOR *SALMONELLA* DETECTION. Although a very diverse assortment of culture conditions have been proposed for the isolation and identification of PT salmonellae, most standard methods follow a general scheme that involves four principal stages. First, nonselective preenrichment is used to encourage the growth of very small numbers of salmonellae or to allow the recovery of injured *Salmonella* cells. Preenrichment is not advisable when testing samples (such as intestinal contents or feces) with large numbers of competing organisms that might overgrow salmonellae in the nonselective broth. Second, selective enrichment is used to allow additional expansion of the *Salmonella* population while suppressing the growth of other organisms. Third, plating on selective agar media is used to obtain isolated colonies, each derived from a single cell. Nonselective agar plating media are also sometimes used with swabs from internal organs. Fourth, colonies with appearances characteristic of salmonellae are subjected to biochemical and serologic tests to confirm their genus and serotype identity. Virtually all proposed methods require the last two of these steps, but enrichment requirements vary according to the nature of the sample.

Tissue samples (except for samples of intestinal tissues or contents) from infected birds generally contain relatively few competing organisms. Swab or loop samples taken from internal organs are often transferred directly to plates of both selective and nonselective agar media, without broth enrichment. Excised tissue samples, and any samples derived from the intestinal tract, are generally transferred initially into selective enrichment broth.

Because fecal contamination may result in the presence of diverse flora, eggshells are generally sampled without preenrichment. The surface of eggshells can be sampled by immersion in selective broth media or the entire shell (including interior structures and shell membranes) can be sampled by aseptic breaking to release the contents followed by manual crushing and addition of selective enrichment broth (108, 104). Before culturing egg contents for contamination by salmonellae, the shell exterior must be disinfected to prevent fecal contaminants of the shell from being transferred to the contents during breaking.

Because of the very low prevalence of salmonellae (primarily *S. enteritidis)* in egg contents, and because *Salmonella* contaminants tend to be present in eggs in very small numbers, the entire liquid contents of 10–20 eggs are often pooled together for sampling to minimize demands on laboratory resources. Egg contents pools are generally incubated before further culturing to allow the *Salmonella* population to expand to a consistently detectable level (106, 115). Iron supplementation of whole egg pools can increase the multiplication of some *S. enteritidis* strains during incubation (76, 115, 116). Preenrichment of egg contents has been shown to lead to a greater sensitivity of *S. enteritidis* detection than direct selective enrichment (105, 291), probably by allowing very small initial levels of salmonellae to expand to levels that will survive the harsher conditions of selective enrichment (50). Direct plating of incubated egg pools onto selective agar media can markedly reduce the time, media,

and labor demands of culturing, but does so at a significant loss in detection sensitivity (105, 115).

Environmental samples are generally collected in sterile plastic bags and cultured by transfer into selective enrichment broth. Litter or fluff samples can be collected from several sites in each house. Various environmental surfaces can be sampled using moistened gauze pads. Similarly moistened drag swabs can be drawn across floor litter or dropping pits. Feed should be tested by collecting several representative samples from each lot and transferring into selective enrichment broth. Preenrichment of poultry feed samples has been reported to be unnecessary or even counterproductive (68, 69, 78).

Culture media are generally incubated for 24 hr at 37 C. Longer (48-hr) incubation in nonselective media has been reported to be useful for recovering small numbers of *S. enteritidis* from egg contents (108, 157). Shorter (6-hr) selective enrichment has been used successfully to recover salmonellae from animal feeds (78), but such abbreviated selective enrichment is likely inadequate to suppress competing microflora in more heavily contaminated samples (79). Incubation of selective enrichment cultures at elevated temperatures (42–43 C) has been recommended to suppress the growth of competing microflora, especially in intestinal samples or samples containing fecal material (80, 82, 205). Delayed secondary enrichment, in which selective enrichment broth cultures are held for an additional 5 days at room temperature to allow salmonellae an extended opportunity to grow to detectable levels, has been found to improve the recovery of PT salmonellae from poultry diagnostic and environmental samples (326, 328).

CULTURE MEDIA. A diverse array of media has been developed and recommended for isolating and identifying salmonellae. Although some evidence has suggested that proper media selection is somewhat contingent upon the type of sample being tested, several media have been consistently effective in a variety of applications. Formulations and preparations for most standard *Salmonella* media are provided in Atlas and Parks (6), and most preparations are commercially available in dehydrated form from several manufacturers.

Suggested broth media for the preenrichment of samples for salmonellae include buffered peptone water and trypticase soy broth. Stephenson et al. (291) reported that, of five preenrichment media tested, trypticase soy broth provided the greatest sensitivity of detection of *S. enteritidis* in artificially contaminated egg yolks.

Selective broth media most often used for isolating PT salmonellae in recent years include tetrathionate (TT) broth, selenite-cystine (SC) broth, and Rappaport-Vassiliadis (RV) broth. Tetrathionate broth preparations have been found to yield a higher frequency of *Salmonella* detection than RV broth or SC broth from a variety of types of samples, including cloacal swabs, intestinal tissues, pooled egg contents, poultry feeds, and various foods (68, 79, 83, 105, 311). Rappaport-Vassiliadis broth has been effectively used to isolate salmonellae from raw chicken and egg contents pools (2, 157, 324).

A large number of agar media are available for the isolation of PT salmonellae. Among the most commonly used plating media are brilliant green (BG) agar, XLD agar, XLT4 agar, bismuth sulfite agar, and Hektoen enteric agar. Brilliant green agar remains the most widely used medium for *Salmonella* isolation from poultry sources and has been shown to be effective in application to diverse tissue, environmental, egg, and feed samples (68, 105, 326, 327). XLT4 agar has been successfully applied to detect salmonellae efficiently from poultry house environmental drag swabs (216). The addition of novobiocin to agar plating media has been demonstrated to improve *Salmonella* recovery by suppressing the growth of some competing organisms (notably *Proteus*) that might otherwise overgrow the salmonellae (295, 296). Samples should always be streaked onto two different media, preferably with dissimilar indicator systems for differentiating salmonellae from other organisms.

CONFIRMATION OF GENUS AND SEROTYPE. Colonies on selective agar plates that have the characteristic appearance of PT salmonellae must be tested further to confirm their genus identity and to determine their serotype. The combined use of triple sugar–iron agar and lysine–iron agar provides an effective presumptive test for identifying PT salmonellae. Additional differentiation of PT salmonellae from other organisms can be accomplished by determining the fermentation pattern of each isolate for a set of six particular carbohydrates, as described by Cox and Williams (67). The serogroup of each isolate can be determined by slide agglutination tests with polyvalent antisera to groups of somatic O antigens, and the serotype can then be determined by slide agglutination tests with monovalent antisera to specific O antigens and tube agglutination tests with antisera to flagellar H antigens.

RAPID DETECTION TECHNOLOGIES. Obtaining negative results from conventional culturing methods for salmonellae requires several days for most types of samples, and confirming positive results adds even more time. Many comparatively more rapid techniques have been proposed and investigated in recent years, but none have yet achieved particularly wide acceptance. Most of the rapid

methods reduce the time requirements of testing by 1 or more days, and many are amenable to some degree of automation. Concerns about rapid methods include their typically high cost, their usual dependence on enrichment to achieve sufficient cell densities to allow detection, and their frequent inability to demonstrate a specificity of detection comparable to that of conventional culturing. Although properties as diverse as the ability to exhibit motility (257) or to cause specific changes in the electrical impedance of media (247) have been successfully used to enrich for or identify salmonellae, most efforts to develop rapid *Salmonella*-detection methods have centered around the use of specific antibodies or DNA probes.

Specific antibodies to *Salmonella* antigens have been used to develop a variety of enzyme-linked immunosorbent assay (ELISA) methods. These tests, using polyclonal antibodies to *Salmonella* LPS or flagella, have been reported to detect salmonellae in eggs, tissues, cloacal swabs, environmental drag swabs, litter, and feed (136, 206, 258). Monoclonal antibodies to outer membrane proteins or flagella have been used as the basis for ELISA tests to specifically detect *S. enteritidis* in eggs, tissues, and environmental samples (172, 173). Although not apparently quite as sensitive as conventional culture methods (296), ELISA tests are usually reported to detect salmonellae at a frequency comparable to standard methods, and to do so at least 1 day sooner. One or more initial enrichment culturing steps, however, are generally necessary to allow the expansion of the *Salmonella* population into the range detectable by ELISA, which is often estimated at between 10^5 and 10^7 salmonellae per mL (29, 136, 172). Like conventional culturing methods, ELISA tests are also somewhat prone to false-positive results from competing flora able to grow in enrichment media (26).

Another application of antibodies for detecting salmonellae involves coating small magnetic beads with specific antibodies. When mixed with the sample to be tested, the antibody-coated beads will bind to any *Salmonella* target antigens present and a magnetic field can then be applied to recover the bead–antibody–antigen complex. In essence, immunomagnetic separation thus serves as an alternative to broth enrichment for concentrating salmonellae, but with the advantages of requiring less time and having no adverse effect on sublethally injured cells. A method using immunomagnetic separation to concentrate salmonellae before plating on selective agar detected a higher frequency of *Salmonella* contamination in samples of poultry meat, tissues, eggshells, and cloacal or fecal swabs than did either traditional selective enrichment or motility-based enrichment (75). Immunomagnetic separation has also been used to detect small levels of *S. enteritidis* contamination in pools of egg contents by both culturing and ELISA (76, 150).

Another approach to rapid testing for salmonellae in poultry, which has received considerable attention in recent years, involves using probes for particular DNA sequences unique to salmonellae. Hybridization of the probe with DNA extracted from the sample indicates a positive result. DNA probes, in both radiolabeled and colorimetric assays, have been applied to the detection of salmonellae in drag-swab environmental samples from poultry houses with a high degree of specificity (131). The sensitivity of detection of salmonellae by DNA hybridization is similar to that of ELISA, and thus generally also requires one or more enrichment culturing steps. Moreover, DNA hybridization assays are often procedurally complex and are rather expensive in comparison to other available methods. The development of polymerase chain reaction (PCR) technology, however, has allowed the specific amplification of particular target segments of DNA, thereby enabling hybridization reactions with probes to detect salmonellae in feces and environmental drag-swab samples with a very high level of sensitivity (51, 52). Carefully chosen DNA probes can be used along with PCR to detect salmonellae with specific characteristics, such as those carrying particular virulence genes (204).

Serologic Diagnosis of Infection. Specific antibodies to PT salmonellae have been detected in the sera of infected poultry with a high degree of sensitivity using several different agglutination and ELISA methods. Detectable serum antibody titers are often still present long after all salmonellae have evidently been cleared from tissues and fecal shedding has ceased (134, 329, 340). Various tests for serum antibodies to salmonellae have been applied effectively for detecting both naturally (47, 151, 252, 323) and experimentally (14, 109) infected poultry. Because antibody tests only demonstrate prior exposure to salmonellae, and do not provide unequivocal evidence of a currently ongoing infection in a flock, positive serologic results must generally be followed by bacteriologic culturing for confirmation. Other problems with serologic testing include the possibility that subclinical infections will lead to fecal shedding without sufficient invasion and dissemination to elicit a detectable antibody response (240), the general immunologic unresponsiveness of very young birds to *Salmonella* infection (329), and cross-reactions between antibodies to similar PT serotypes (235).

The various agglutination tests have been applied successfully for detecting chickens naturally or experimentally infected with *S. enteritidis* or *S. typhimurium* on many occasions (48, 109, 151, 252, 341). The principal agglutination test formats in-

clude rapid whole-blood plate, serum plate, tube agglutination, microagglutination, and microantiglobulin tests. All of these tests rely on the ability of specific antibodies to cause visible agglutination when mixed with antigen preparations of killed whole *Salmonella* cells. Except for the tube test, all agglutination assays use stained antigens to improve the ease of visualization of the agglutination reaction. The rapid whole-blood plate test is the most widely used method for detecting antibodies to *S. pullorum* or *S. gallinarum* (317). Tube agglutination tests are used extensively for confirming rapid whole-blood plate test results for *S. pullorum* and *S. gallinarum* (317), but have not been widely applied to detecting PT salmonellae.

Microagglutination tests for PT salmonellae are conducted in 96-well disposable plastic plates (317). Microantiglobulin tests enhance the sensitivity of microagglutination tests by using an additional incubation period with a secondary antibody directed against chicken immunoglobulins to increase the overall agglutination of the target antigen (339). The microantiglobulin test has frequently been reported to provide greater sensitivity for detecting PT infections than other agglutination test methods (53, 235, 342, 341).

Paratyphoid salmonellae infections in poultry have also been effectively detected using various ELISA approaches. For example, ELISA tests with LPS, flagella, or outer membrane proteins as antigens have been successfully used to identify chickens infected naturally or experimentally with *S. typhimurium* or *S. enteritidis* (14, 174, 233, 235, 310). By using very precisely defined antigens, ELISA tests often achieve a high degree of specificity and are thus frequently associated with fewer false-positive results due to cross-reactions between serotypes than are agglutination reactions (134, 174, 322). Screening for serum antibodies using a flagella-based ELISA test has been used satisfactorily for detecting *S. enteritidis* in Dutch breeder flocks (323).

Antibodies deposited in egg yolks can also be used to detect poultry infected with PT salmonellae. Both microantiglobulin (111) and ELISA (14, 77, 234) tests have been applied to find antibodies to *S. enteritidis* and *S. typhimurium* in eggs from naturally and experimentally infected chickens. Gast and Beard (111) reported that the presence of specific antibodies in eggs from commercial laying flocks in the United States was directly correlated with the presence of *S. enteritidis* in tissue samples from those flocks. Van de Giessen et al. (320) found a direct relationship between specific egg-yolk antibody titers and the incidence of shedding of *S. enteritidis* in the feces of laying flocks in the Netherlands.

PREVENTION AND CONTROL. Efforts to establish critical control points for preventing PT infections in poultry are handicapped by the diversity of sources from which salmonellae can be introduced into flocks or houses. Effective prevention and control programs, therefore, must involve coordinated and simultaneous attacks on the problem from several directions. Eggs and chicks or poults should be secured only from demonstrably *Salmonella*-free breeding flocks. Hatching eggs should be properly disinfected and hatched according to stringent sanitation standards. Poultry houses should be thoroughly cleaned and disinfected by recommended procedures between flocks. Rodent and insect control measures should be incorporated into house design and management and verified by periodic testing. Rigidly enforced biosecurity practices should be implemented to restrict entry onto poultry housing premises to only authorized personnel and equipment, and to prevent horizontal transmission of salmonellae between houses. Only pelleted feed or feed containing no animal protein should be used, to minimize the likelihood of using contaminated rations. Treatments such as medication, competitive exclusion cultures, or vaccination can be applied to reduce the susceptibility of birds to *Salmonella* infection. Finally, the *Salmonella* status of poultry and their environment should be monitored by frequent testing. Such multifaceted prevention and control programs have reportedly been successful in addressing *Salmonella* problems in both chickens (90, 215) and turkeys (248).

Increased international interest in controlling PT infections, especially *S. enteritidis*, has led to the development and implementation of many testing and monitoring programs in recent years (3). In the United States, the National Poultry Improvement Plan (NPIP) defines stringent sanitation and testing standards for breeder flocks to prevent the transmission of *S. enteritidis* infection to egg-laying stock (317). Participation in this plan requires compliance with standards for feed selection and handling, disinfection of hatching eggs, and hatchery sanitation. The NPIP testing for *S. enteritidis* involves bacteriologic monitoring of the environment and serologic monitoring of birds, with culturing of tissues from selected birds used for confirmation. A similar protocol, involving screening for infection with drag-swab samples of the manure pit and confirmation of infection by sampling tissues from selected hens, has been used by the USDA to test epidemiologically implicated laying flocks for *S. enteritidis* (315). More recent programs to assure the microbiologic safety of eggs have continued the use of environmental sampling as a screening device, but instituted egg culturing as the confirming step in place of culturing tissues (316).

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Medication. Although medication is often used to prevent or treat PT infections, the efficacy and wisdom of using this approach are still topics of considerable debate. Antibiotics were used effectively both as therapeutic and prophylactic agents as part of control efforts for *S. enteritidis* in broiler and broiler breeder flocks in Northern Ireland (215). Combined administration of polymyxin B sulfate and trimethoprim to chicks both prevented and cleared experimental infections with *S. enteritidis* (122). Various antimicrobial agents, including tetracyclines, neomycin, bacitracin, and sulfa drugs (except in laying chickens) are approved and regularly used in poultry (225). Injectable gentamicin and spectinomycin are approved for use in controlling yolk sac infections acquired at the hatchery (225), especially in turkey poults.

Williams and Whittemore (343) reported that adding any of five different antimicrobial agents to the drinking water of chicks reduced the frequency of isolation of subsequently administered *S. typhimurium* from cloacal swabs. However, as birds removed from antimicrobial treatment were found to be active carriers of salmonellae, the investigators concluded that drug excretion was often interfering with recovery of the infecting organism from fecal material, and thereby resulting in a misleading impression that treatment was efficacious. Olesiuk et al. (241) similarly found that five antimicrobial agents had only very limited value for preventing or eliminating experimental *S. typhimurium* infection. The administration of some antibiotics has been reported to increase the susceptibility of poultry to *Salmonella* infection, perhaps by suppressing the growth of other microflora capable of exerting inhibitory activity against salmonellae (207, 208). Antibiotics are also sometimes added to poultry feeds at subtherapeutic levels to promote growth. Both therapeutic and subtherapeutic antibiotic administration has been shown to select for drug-resistant strains of salmonellae, thereby potentially compromising the effectiveness of those drugs in both animals and humans (117, 118, 177). Multiple resistant salmonellae, insensitive to the effects of several antimicrobial agents, have become increasingly prevalent among poultry isolates in both the United Kingdom and North America (81, 305).

Competitive Exclusion. Newly hatched chicks and poults are highly susceptible to infection by PT salmonellae, but quickly become more resistant. This age-associated decrease in susceptibility to salmonellae is largely attributable to the acquisition of protective intestinal microflora from the environment. The evident ability of other intestinal bacteria to exert inhibitory effects against salmonellae has served as the basis for the development of a diverse group of treatments often referred to collectively as competitive exclusion (CE). Competitive exclusion treatments involve administering defined or undefined bacterial cultures to poultry in order to diminish intestinal colonization by salmonellae.

The efficacy of CE treatment has been illustrated repeatedly in both chickens and turkeys, using intestinal or fecal material from mature birds or undefined anaerobic cultures derived from such material. Administration of CE cultures has been shown to diminish both intestinal colonization by various PT salmonellae and subsequent invasion to internal tissues (238, 259, 268, 283, 284). Used litter can also be used as a source of CE cultures (62, 63). In field trials in commercial broiler chicken flocks in several nations, treatment with CE cultures led to significant reductions in the incidence of salmonellae in live birds and on carcasses (32, 124, 333, 334). After antibiotic therapy to treat *Salmonella* infections in replacement pullet flocks, administration of a CE preparation was used effectively for providing a complete intestinal microflora to prevent reinfection with salmonellae (170). In some instances, treatment with CE cultures has been observed to enhance the clearance of preexisting *Salmonella* infections (330, 352).

Competitive exclusion cultures have been shown to be effective against salmonellae following administration to poultry in a variety of forms, including crop gavage, application to the vent lip, whole-body spraying or droplet application, addition to drinking water, encapsulation in lyophilized alginate beads added to the feed, and in ovo inoculation into the air cell (64, 65, 72, 267). Considerable research has sought to identify the microflora constituents responsible for protection against salmonellae. A defined mixture of microorganisms would likely produce a given level of protection with greater consistency than undefined cultures and would also provide a greater assurance of safety than is available with mixtures of unknown organisms. The protective efficacy of mixtures of small numbers of intestinal bacteria is usually very limited (289), but more diverse defined mixtures can provide significant protection (66, 121, 236, 289).

Several factors have been identified that affect the overall usefulness of CE cultures for controlling PT salmonellae infections in poultry. Although CE treatment generally reduces the incidence of intestinal colonization by salmonellae, it does not prevent it altogether. Moreover, the protective efficacy of CE cultures can sometimes be overcome by severe challenge with salmonellae (283). Administration of CE cultures can thus contribute significantly to an overall *Salmonella* control effort, but proper cleaning and disinfection, biosecurity, rodent reduction, and other similar measures are still necessary

were cultured from only 2% of 173 submissions not corresponding to the profile for AIH/AVH. Both brilliant green agar and blood agar were suitable to culture VLOs from bile, and yielded a 32% recovery rate, compared with 35% using embryos.

Studies on field isolates derived from laying flocks in Ontario showed that VLOs isolated from either bile or cecal contents of specific birds showed identical biochemical and serologic criteria. It was also noted that VLOs derived from various submissions showed differences in pathogenicity and ability to colonize the cecum (133). In a recent study on the prevalence of *C. jejuni* in broilers from 44 farms sampled at the time of processing, 21% of 223 livers showing gross necrotic lesions yielded three biotypes of *C. jejuni*. In comparison, an isolation rate of 12% was obtained from 50 unaffected livers, with biotype 2 predominating. These observations would not, however, support the authors' contention that *C. jejuni* was responsible for hepatic lesions (16).

In assessing the literature relating to the AIH/AVH complex, it is evident that a field syndrome in commercial laying hens occurred prior to and during the mid-1960s. The syndrome was characterized by low morbidity and mortality with degeneration of the liver as the principal diagnostic feature. The current enigma facing pathologists and epidemiologists is the complete disappearance of the condition as a clinical entity from the United States and western Europe.

Studies conducted during the past few years have failed to reproduce hepatopathy using strains of *C. jejuni* derived either from humans or avian species (103, 104). It is noted that only enteritis, characterized by diarrhea, can be induced by infecting newly hatched chicks (141) or poults (69), as well as mammalian food animals (30), exotics (74), and companion species (31).

Necrotic hemorrhagic hepatitis-splenomegaly of commercial laying hens has been described in Canada and regions of the United States. As yet, no etiology has been defined, but *C. jejuni* has been isolated from livers of affected birds (99).

There are two speculative explanations for the disappearance of the AIH/AVH complex. The original condition may have been caused by a pathogen other than the VLO that was isolated; however, the experimental reproduction of the condition with an agent cultured on artificial media tends to disprove this hypothesis (93). It is more probable that the VLO interacted synergistically as an opportunist with some other pathogen (142), analogous to the association between *C. jejuni* and parvovirus in dogs (34) or with immunosuppressive or debilitating agents in humans (77). The primary pathogen or cofactor may have subsequently been eliminated by comprehensive immunization programs introduced during the mid-1960s. There is no conclusive experimental evidence to indicate the VLOs isolated from cases of AIH/AVH complex were in fact campylobacters. Attempts in 1985 to propagate and characterize the agent recovered from frozen lyophilized yolk material stored since 1960 were unsuccessful (21).

CAMPYLOBACTERIOSIS

ETIOLOGY. Campylobacteriosis is attributed to infection by thermophilic members of the genus *Campylobacter* (107). The three species of clinical significance, *C. jejuni*, *C. coli*, and *C. laridis*, are microaerophilic, gram-negative, spiral, uniflagellate organisms, which demonstrate characteristic darting motility when examined under dark-field illumination (118).

Classification. The nomenclature of the genus *Campylobacter* has been subject to frequent changes in response to emerging biochemical and taxonomic criteria. Various classification schemes for the genus have been described (40, 60) that clearly designate *C. jejuni*, the predominant organism isolated from avian hosts, as a separate and valid species (121), and not a subspecies of *C. fetus* as in early literature. The phylogeny of the campylobacters has been evaluated on the basis of 16S ribosomal ribonucleic acid sequencing, with *C. jejuni*, *C. coli*, and *C. laridis* included in Homology Group I (132). *Campylobacter jejuni* is the most frequently occurring member of the thermophilic triad, but *C. coli* may occasionally be isolated from the intestinal tract of poultry and derived meat products (84, 102). *Campylobacter laridis*, previously referred to as an NARTC (nalidixic acid-resistant thermophilic campylobacter) (7), is the remaining species in the related thermophilic group and is isolated mainly from free-living marine birds such as gulls (*Larus* spp.) (58).

Morphology and Staining. Campylobacters are spirally curved rods which appear S-shaped or

in "gull-wing" forms, and range in size from 0.2 to 0.8 μ in diameter and 0.5 to 6.0 μ in length. All species are motile and possess a single polar flagellum, although bipolar cells are occasionally observed (120). Campylobacters are gram-negative, but require a fuchsin-based counterstain because of their relative inability to take up saffranin (106).

Growth Requirements. The campylobacters of clinical significance show optimal growth on artificial media at 43 C (118), although minimal growth occurs at 37 C.

Campylobacters are microaerophilic, and satisfactory propagation requires an atmosphere comprising 5% oxygen, 10% carbon dioxide, and 85% nitrogen (98). Purchase of a commercial gas mixture is recommended for laboratories conducting routine isolation of campylobacters. An analysis of alternative methods to achieve a microaerobic environment, including commercial gas packs, torbal and candle jars, or application of Fortner's principle, has demonstrated various disadvantages relating to decreased growth, extended incubation periods, or high cost (44).

Appropriate methods of transport and storage of campylobacters are necessary because these organisms are sensitive to desiccation. An enriched semisolid brucella medium incorporating 10% ovine blood can be used to maintain viability of cultures for transport at 25 C for up to 3 wk (139). Six alternative transport media were compared in a structured study of *C. jejuni* survival. Cary-Blair medium with decreased agar content was superior to Stuart's medium for storage periods exceeding 7 days at 25 C (76). Both Stuart's fluid medium and Cary-Blair semisolid transport medium are commercially available in plastic tubes with accompanying rayon-tipped swabs to facilitate sampling of biologic material for subsequent submission to a diagnostic laboratory.

During the late 1970s, selective media containing antimicrobial compounds were introduced, simplifying the isolation and propagation of campylobacters (91). Commercial media contain brucella agar, blood agar base, ovine, bovine or equine blood, and various antibiotic additives, including bacitracin, novobiocin, trimethoprim, actidone, cyclohexamide, cephalothin, and colistin. Differences in source of blood and antibiotic content of media have been evaluated under field and laboratory conditions. Medium BU-40 was shown to be superior to both Skirrow's and Butzler's media in terms of efficiency of isolation of *C jejuni* (82). Preston medium, a selective agar incorporating lysed horse blood and antibiotics yielded higher isolation rates of *C. jejuni* than Skirrow's, Butzler's, Blaser's, or Campy-BAP media. Recovery can be enhanced by preincubation in Preston enrichment broth (15). A semisolid medium has been developed for transport and enrichment of fecal specimens, which enhances the rate of recovery of *C. jejuni* from patients receiving antibiotic therapy and from their contacts (19). A blood-free selective medium containing charcoal has been shown to be as effective in isolating *C. jejuni* as conventional Skirrow's medium (62). Campy-Choc Agar, a charcoal- and blood-free medium, compared favorably with three conventional media which yielded a 0.3% *C. jejuni* isolation rate in a survey of 2890 human fecal specimens (137).

The current status of isolating enteric pathogens, including campylobacters, has been extensively reviewed with specific reference to selection and preparation of samples, preenrichment, and selective plating. Despite the introduction of immunoassays and gene probes, conventional media provide acceptable selectivity and inhibition for diagnostic and survey purposes (38).

Colonial Morphology. The incubation period for detecting colonial growth generally exceeds 24 hr. With a low concentration of organisms in the inoculum, or when an inhibitory medium is used, incubation for up to 72 hr may be required to observe colony formation (82). On primary isolation, colonies may be either flat, translucent, and gray with a tendency to coalesce, or be raised, opaque, and brown-gray with discrete margins (120). The presence of swarming colonies is attributed to higher moisture content of freshly prepared media in contrast to the discrete colonies formed on media that has aged for a few days prior to inoculation (17). Colonies are nonhemolytic on blood agar (121).

Biochemical Properties. As campylobacters are unable to ferment carbohydrates, energy is derived from the degradation of amino acids. The three thermophilic species of clinical significance all reduce selenite, are oxidase and catalase positive, and indole negative (118). Differentiation between *C. jejuni*, *C. coli*, and *C. laridis* is based on nalidixic acid sensitivity and hippurate hydrolysis (Table 10.1). The additional properties of DNA hydrolysis and rapid production of hydrogen sulfide were incorporated into an extended biotyping scheme for the three species (Table 10.2).

An evaluation of various biochemical characteristics of 264 cultures permitted differentiation between eight species or subspecies of *Campylobacter* (51, 71).

Other sources have defined up to eight biotypes of *C. jejuni* based on hydrolysis of DNA and hippurate, and growth on charcoal-yeast extract agar (51). Details concerning biochemical reactions of the thermophilic campylobacters have been com-

Table 10.1. Differentiation among catalase-positive *Campylobacter* species according to biochemical characteristics

Species	Growth at 25 C	Growth at 42 C	Nalidixic acid sensitivity[a]	Hippurate hydrolysis
C. fetus	+	–	R	–
C. coli	+	+	S	–
C. jejuni	–	+	S	+
C. laridis	–	+	R	–

Source: (119).
[a]R = resistant; S = sensitive.

Table 10.2. Biotyping scheme for *Campylobacter jejuni*, *C. coli*, and *C. laridis*

	C. jejuni				*C. coli*		*C. laridis*	
Test	I[a]	II	III	IV	I	II	I	II
Hippurate hydrolysis	+	+	+	+	–	–	–	–
Rapid H_2S test	–	–	+	+	–	–	+	+
DNA hydrolysis	–	+	–	+	–	+	–	+

Source: (71).
[a]Biotype.

prehensively reviewed with specific reference to differential characteristics to distinguish between field isolates (40, 118, 121).

Resistance to Physical and Chemical Agents and Antibiotics

PHYSICAL AGENTS. Campylobacters are extremely sensitive to desiccation. A suspension of *C. jejuni* impregnated onto a filter paper strip will not survive beyond 2 hr at 20 C (76). Infectivity is retained for up to 4 wk in water at 4 C (9), but *C. jejuni* can remain viable in milk for 3 wk at 4 C and for 24 hr at 25 C (100).

A comprehensive study on the survival of *C. jejuni* in biologic systems showed the organism could multiply in bile stored for 2 months at 37 C, but was rapidly destroyed in human urine at the same temperature. At 4 C, *C. jejuni* retained viability for 3 wk in feces and 5 wk in urine (10). *C. jejuni* persisted for a 10-day period on chicken portions stored at either −9 C or −12 C, and contamination could be detected after 182 days storage at −20 C (145).

Lyophilized cultures of *C. jejuni* in Brucella broth containing 0.16% agar and blood retain viability for many years. When cryoprotective agents such as dimethyl sulfoxide or glycerol are added to heavy suspensions of the organism in brucella broth, survival exceeds 3 yr at −80 C (121).

Irradiation pasteurization at a dose of 1.0 kGy from a cobalt-60 source effectively eliminated *C. jejuni* surface contamination at a level of 10^3 colony-forming units (CFU)/cm^2 (146).

CHEMICAL RESISTANCE. The in vitro sensitivity of *C. jejuni* to various disinfectants was assessed using three strains of the organism isolated from diarrheic human patients. A 1:200,000 solution of 5% sodium hypochlorite and a 2.5% solution of 10% formaldehyde both destroyed *C. jejuni* within 15 min. Contact with 0.15% organic phenol, a 1:50,000 quaternary-ammonium compound, or 0.125% glutaraldehyde killed a 10^7 CFU suspension of *C. jejuni* within 1 min (140). Resistance to chemical agents is increased by the protective action of biological material. In a comparative study of the efficacy of chemical disinfectants used in the food industry, it was shown that 3% succinic acid, 0.5% glutaraldehyde, and 25 ppm poly-(hexamethylenebiguanide hydrochloride) were all able to reduce significantly the level of *C. jejuni* contamination on the surface of chicken drumsticks. Chlorine levels below 120 ppm were ineffective under conditions simulating immersion in poultry processing plant tanks (146).

ANTIBIOTIC SENSITIVITY. The antibiotic sensitivity of the three thermophilic campylobacters has been extensively documented (61, 121). A study conducted in Sweden showed close similarity in antibiotic sensitivity between approximately 75 isolates derived from diarrheic human patients and from processed broilers. The majority of isolates were sensitive to erythromycin and doxycycline, although a high proportion of strains were resistant to the tetracyclines, and an acceptable response to gentamicin, chloramphenicol, and carbenicillin was obtained (129). Similar results were achieved in an investigation involving 276 food animal isolates, including 107 derived from chickens and 403 human fecal isolates from patients admitted to a hospital in Brussels. Furazolidone was shown to be the most effective compound, with most human and animal isolates also sensitive to erythromycin and gentamicin. Approximately 26% of the chicken strains were resistant to tetracycline (136). In evaluating the efficacy of 16 antimicrobial compounds against 103 clinical isolates of *C. jejuni*, kanamycin and gentamicin were shown to be completely effective, in contrast to tetracycline, penicillin G, and erythromycin. Approximately 38%, 36%, and 13% of the isolates were resistant to these three compounds, respectively (79). The biochemical mechanisms and genetic aspects of antibiotic resistance in campylobacters have been comprehensively reviewed in relation to quinolones, tetracyclines, aminoglycosides, and macrolides (131).

Serotyping. A significant advance in serotyping *C. jejuni* was achieved with the introduction of the Penner scheme based on soluble, heat-stable "O" antigens derived from surface lipopolysaccharides (95). Antisera produced in rabbits can be ap-

plied to a passive hemagglutination technique to identify 60 serotypes of *C. jejuni*. Subsequent studies showed that *C. jejuni* and *C. coli* have individual antigens, with minimal commonality between species (96). The alternative Lior serotyping scheme is based on heat-labile "H" antigens (72). This system is read using slide agglutination and requires multiple absorption of heterogenous antisera. In a comparison between the two schemes, the Penner technique was found to be marginally more specific than the Lior system and, although requiring more equipment, was faster under practical conditions in a diagnostic laboratory (92).

Bacterial restriction endonuclease DNA analysis (BRENDA) has been used to differentiate campylobacters. An epidemiologic study has demonstrated that 50% of a sample of 316 isolates of *C. jejuni*, representing 11 of 60 BRENDA types, were common to both humans and poultry (57).

PATHOGENESIS AND EPIZOOTIOLOGY

Natural Hosts. Poultry serve as primary reservoir hosts of thermophilic campylobacters (41). Up to 90% of broilers may be infected (8), while 100% of turkeys (70, 74, 76) and 88% of domestic ducks (97) may harbor the organisms.

Various species of *Campylobacter* have been isolated from free-ranging pigeons in the United States (75) and Japan (65). Infection has been recorded among game birds, including partridges, pheasants (138), and quail (80). Campylobacters have been isolated from marine birds such as puffins (59) and gulls (58), from waders (39), and migratory Anseriformes (89). Approximately 8% of samples taken from eight species (Columbiformes and Passeriformes) in a Japanese investigation yielded *C. jejuni*. It was noted that numerically higher recovery rates were obtained from scavengers and omnivores than from granivores (54). The prevalence of *C. jejuni* in avian species is a function of the intensity of surveillance, since diligent collection and culturing will generally reveal intestinal infection in many orders of exotic and domestic birds within a specific area (3, 147).

Experimental Hosts. The wide range of laboratory animal species susceptible to *C. jejuni* includes rabbits, mice, rats, hamsters, and primates (33). Animal models for campylobacter enterocolitis in humans include mice (13), hamsters (35), and ferrets (36).

Campylobacters can be propagated in vitro in tissue culture systems, including Chinese hamster ovary cells (45), HeLa cells (28), and human epithelial cell lines (18). Fertile chicken eggs serve as a convenient system for isolation and propagation of campylobacters. Both *C. jejuni* and *C. coli* infection of embryos can be achieved by either the chorioallantoic route or by direct intravenous injection on the 11th day of incubation (29). The embryo system can be used as a model to differentiate between the relative virulence of various strains of *C. jejuni* and *C. coli* derived from cases of human and animal enterocolitis. Fecal isolates of *C. jejuni* obtained from chickens and turkeys are lethal when introduced via the yolk sac route into embryonated eggs of the corresponding species (69).

Transmission. Despite the fact that C. jejuni is prevalent as an intestinal commensal in floor-housed turkeys, broiler breeders, and layer-type breeder chickens, there is no evidence to show that campylobacters can be transmitted vertically by either transovarian infection or by penetration of the egg shell after oviposition. An extensive survey failed to demonstrate *C. jejuni* in fertile turkey eggs and poults derived from a flock known to carry the organism (1). Another study showed that *C. jejuni* did not penetrate the shells of eggs produced by cage-housed hens, despite recovery of the organism from the intestinal tract and feces (27). Infrequent isolation of *C. jejuni* from the inner and outer membranes of refrigerated eggs is attributable to shell damage. Failure to demonstrate *C. jejuni* within or on the surface of table eggs was confirmed in field studies conducted on three farms in Louisiana (114) and on 23 units in New York (6). It is possible to induce egg-penetration by immersion in a suspension of *C. jejuni* (86) or by using either temperature or pressure differential techniques (22). It is concluded that under practical commercial conditions, desiccation will destroy organisms on the surface of clean eggs within a short period following oviposition. Artificial contamination of eggs with a fecal suspension of *C. jejuni* showed that viability did not exceed 16 hr, and that 50% of the artificially contaminated egg shells were free of viable campylobacters within 10 hr (114). Rejection of grossly soiled eggs, physical removal of small quantities of fecal material adherent to the shell surface, and fumigation or chemical disinfection within 2 hr of collection will all reduce the possibility of egg-borne transmission of campylobacters (115).

Experiments have conclusively demonstrated that contamination of feed and water by chronic intestinal carriers transmits *C. jejuni* to susceptible contacts (81). This study also showed that intestinal infection persisted for at least 63 days in broilers housed on wire-mesh floors, which prevented coprophagy.

Houseflies (*Musca domestica*) can acquire *C. jejuni* from contaminated litter and are capable of transmitting infection to susceptible chicks under controlled experimental conditions (113). A field investigation that revealed 50% of houseflies in the vicinity of a poultry farm were infected with *C. jejuni* (101) and the recovery of the organism from

cockroaches (135) imply that insects may play a role in transmission of campylobacteriosis.

The presence of *C. jejuni* in the feces of domestic sparrows captured in a turkey house suggests the role of free-living birds in introducing infection into commercial poultry flocks (1, 122).

Surveys on broilers (85) and turkey flocks (1) showed that chicks and poults remain uninfected for up to 3 wk when placed into thoroughly disinfected houses containing new litter. Both *C. jejuni* and *C. coli* can be introduced into houses by nonconfined companion animals, vermin, and footwear contaminated with feces and litter (4). Campylobacters are spread rapidly within flocks by horizontal, fecal-oral infection. Consumption of fecally contaminated feed and litter and nonchlorinated water dispensed from trough-type drinkers contribute to dissemination of the organism (41). Poultry strains of *C. jejuni* have a marked capacity to spread horizontally among chicks in hatchers during the last 24 hr of incubation, and with subsequent posthatch processing. Artificial infection of one chick in a hatcher resulted in recovery of *C. jejuni* from 70% of the intestines of contact chicks after 24 hr (23).

Incubation Period. *Campylobacter jejuni* colonized the intestinal tracts of 62% of a batch of susceptible day-old broiler chicks within 24 hr of administration of either 10^2 CFU by the intracloacal route, or 10^4 CFU instilled into the crop. The proportion of chicks yielding *C. jejuni* on cloacal swabs increased to 88 and 97%, respectively, on the 3rd and 4th days postinfection (116). In Japanese quail, *C. jejuni* could be recovered from feces (4 CFU/g) 1 day after receiving an oral dose of 10^8 CFU (78).

Clinical Signs. The severity of clinically detectable changes, usually confined to depression and diarrhea, is dependent on infective dose, strain of *C. jejuni* or *C. coli*, and age of the host. Concurrent environmental stress factors or intercurrent disease and immunosuppression may exacerbate the pathogenicity of *C. jejuni*.

A pathogenic, invasive strain of *C. jejuni* isolated from diarrheic human patients in Mexico produced diarrhea in 88% of a batch of day-old chicks which received 10^8 organisms orally. Within 24 to 72 hr, affected chicks showed depression, fecal saturation of the vent plumage, and watery droppings, which persisted for 8 days. Mortality of 32% was recorded in infected chicks from which *C. jejuni* could be isolated from the heart blood and intestinal tract (103). Infection of holoxenic (conventionally-reared) chicks with *C. jejuni* in a trial designed to investigate competitive exclusion, resulted in transient diarrhea (123). Similar observations were made in broiler chicks that were inoculated with 10^3 to 10^6 CFU of *C. jejuni* derived from diarrheic patients in Bangladesh (104).

In contrast, experimental infections have not produced any clinical abnormalities in broiler chicks aged either 2-3 days or 3 wk, although intestinal colonization was achieved by inoculation via both the oral and cloacal routes (116). In a comparison of age susceptibility, diarrhea was induced in chicks within 12 hr of hatch compared with birds 3 days of age which were unaffected by an oral dose of 10^9 CFU. Signs of *C. jejuni* infection included diarrhea, characterized by the presence of mucus and blood, commencing 6 hr after inoculation and extending for 10 days. Recurrence of diarrhea was noted in the subjects housed on raised wire-mesh floors, which inhibited coprophagy (141).

C. jejuni isolated from feces of turkeys produced transient foamy diarrhea and depressed 21-day body weight in poults infected at either 2 or 4 days of age with 5×10^6 CFU by the oral route. In contrast, chicken-origin *C. jejuni* was apathogenic when introduced into 2- and 3-day-old chicks (69). Intestinal colonization of non–clinically affected broilers may be influenced by genetic factors presumably associated with the major histocompatibility complex controlled by immune-response genes designated "Gregion" (128).

Gross Lesions. The principal change associated with *C. jejuni* infection in chicks comprises distention of the intestinal tract extending from the distal duodenal loop to the bifurcation of the ceca. Accumulation of mucus and watery fluid occurs (104), and depending on the cytotoxic properties of the *Campylobacter* involved, hemorrhages may be present (141), consistent with observations in human campylobacteriosis (66).

The presence of red or yellow mottling of the liver parenchyma was noted in newly hatched chicks subjected to contact infection by toxigenic and invasive strains of *C. jejuni* during the last 24 hr of incubation (23). This observation may relate to an experiment in which focal hepatic necrosis was induced in 60% of a batch of experimentally infected chicks which received the immunosuppressive agent cyclophosphamide. Untreated control chicks infected with *C. jejuni* failed to show liver lesions (124). It is likely that an intact and functional immune system is required to prevent dissemination of the organism from the intestinal tract (14).

Histologic Lesions. Histologic changes attributed to *C. jejuni* infection include congestion and mononuclear cell infiltration of the lamina propria and destruction of mucosal cells in the entire intestinal tract. Edema of the mucosa was noted in

the ileum and ceca, with accumulation of mucus, erythrocytes, mononuclear cells, and a few polymorphonuclear cells in the lumen. Within 48 hr of infection, hyperplasia and villous atrophy were evident in the distal jejunum. Electron microscopy revealed the presence of campylobacters within and between cells of the epithelium and lamina propria (141).

In mild cases characterized by distention of the jejunum, microscopic changes were confined to submucosal edema with gram-negative curved rods adherent to the brush border and within enterocytes (104).

DIAGNOSIS. Thermophilic campylobacters can be isolated from feces, and cecal and jejunal contents. With systemic infection, the organism can also be recovered from liver tissue, bile, and blood. Because of the sensitivity of campylobacters to desiccation, special precautions are required when submitting fecal or other biologic material to a diagnostic laboratory. It is advisable to sample using a commercially available transport system comprising a rayon-tipped swab, which is inserted into a tube containing Cary-Blair medium. Bile samples can be obtained by direct aspiration from the gallbladder using a sterile tuberculin syringe.

Isolation of thermophilic campylobacters requires incubation of cultures for 48–72 hr at 43 C in a microaerobic atmosphere. Selective media are required to suppress the growth of contaminants in fecal and other biologic samples (91). Differentiation between *C. jejuni*, *C. coli*, and *C. laridis*, and their biotypes, can be achieved applying the criteria of incubation temperature, nalidixic acid sensitivity, hippurate hydrolysis, and hydrogen sulfide production (71, 119). A serotyping scheme, such as the Penner system, can be used to identify specific organisms for epidemiologic investigations (96).

A 45-kD outer-membrane protein of *C. jejuni* can be detected in cultures of the organism by applying an oligonucleotide probe in a dot-hybridization assay (67).

PUBLIC HEALTH SIGNIFICANCE. Human campylobacteriosis is a food-borne condition of emerging significance (11, 24, 110, 112). During 1984, the *Campylobacter* isolation rate in the United States attained 4.9/100,000 population, with *C. jejuni* representing 99% of the species cultured (130). This estimate grossly understates the actual prevalence of campylobacteriosis, which may be responsible for 2.1 million cases annually in the United States (83). Projections of cost associated with diagnosis and treatment of human campylobacteriosis, including lost productivity and deaths, range from 700 million to 1400 million dollars per annum.

Early studies on the epidemiology of intestinal campylobacteriosis in human populations demonstrated that consumption of chicken meat was a significant risk factor (12, 87, 117). The high carriage rate of campylobacters in the intestinal tract of broilers (47) and turkeys (73) contributes to contamination during processing (42). This is reflected in high levels of *C. jejuni* on poultry meat (90). Recovery of campylobacters from chicken carcasses is approximately six times higher than from pork or beef, and ranges from 30% to 100% of specimens surveyed (125).

The association of campylobacters with poultry meat represents a significant potential for human food-borne infection under conditions of defective handling, inadequate refrigeration, and improper preparation (25, 53). The correlation between specific *C. jejuni* and *C. coli* serotypes in poultry and in diarrheic humans has been documented, with Penner groups 2, 5, 7, 9, and 22 predominating (5). The staff of poultry processing plants are exposed to campylobacteriosis by handling contaminated material, and the condition may be regarded as an occupational disease (46, 56). An outbreak of *Campylobacter* enteritis in Sweden involved 71% of a group of 24 temporary workers who became ill within 2 wk of commencing employment in a poultry plant. In contrast, only 30% of the long-term employees were infected (20). The dynamics of *Campylobacter* contamination of poultry meat and its relationship to human intestinal infection have been extensively documented following completion of a comprehensive epidemiologic study conducted in King County, Washington (49, 50).

Based on field surveys showing a low prevalence of egg shell contamination with *C. jejuni*, and the sensitivity of the organism to desiccation and approved industrial egg-washing compounds, it is unlikely that campylobacteriosis is attributable to consumption of commercially produced table eggs (55).

PREVENTION AND CONTROL. It is impractical to apply preventive action to reduce *Campylobacter* infection in broiler flocks reared on litter. Although extreme biosecurity measures may limit the introduction of campylobacters into breeding farms, current practices in the U.S. broiler industry contribute to infection before depletion. Under commercial conditions, unrestricted movement of personnel, recycling of litter, and the use of earth-floor convection-ventilated housing subject to ingress by flies, vermin, and possibly wild birds, all contribute to colonization of the intestinal tract with *C. jejuni* and *C. coli* (112). Since coprophagy ensures rapid horizontal spread within a flock (81), the expedient of multitier mesh-floor growing would be required to reduce or obviate transmission. Thor-

ough decontamination, including removal of litter and disinfection of equipment and buildings, followed by a rest period of at least 7 days, will effectively eliminate residual campylobacters in poultry housing (37).

Despite early studies showing the inhibition of *Campylobacter* colonization of the intestinal tract by competitive exclusion flora (124), recent trials have shown variable results in reducing infection rates (116). Defined cultures comprising *Citrobacter diversus*, *Klebsiella pneumonia*, and *Escherichia coli* in combination with 2.5% dietary mannose significantly reduced intestinal colonization rates, but did not eliminate infection (105). These findings are attributed to the association of *C. jejuni* with the intraluminal mucin layer and failure of the organism to adhere to enterocytes (127).

Although it is unrealistic to achieve complete elimination of *Campylobacter* infection during the growing period, it is possible to ameliorate processing plant contamination by disinfecting transport coops and by withholding feed for at least 8 hr prior to flock depletion. Postprocessing decontamination of carcasses and portions with chemical solutions will reduce the level of *C. jejuni*. A 0.5% acetic or lactic acid rinse effectively limits levels of viable organisms under controlled laboratory conditions (126). Subsequent studies have shown that 120 ppm chlorine, warm succinic acid, and 0.5% glutaraldehyde all reduced *C. jejuni* contamination of drumsticks (146). Gamma radiation of poultry meat at subradicidation (pasteurization) levels of 1–5 kGy using a cobalt-60 source will eliminate campylobacters without inducing any undesirable organoleptic or biochemical changes in product (68).

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NECROTIC ENTERITIS

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HISTORY, INCIDENCE, AND DISTRIBUTION. Necrotic enteritis (NE) in domestic chickens was first described by Parish in 1961 (54, 55, 56), who reproduced the disease with a strain of *Clostridium welchii* (*C. perfringens*). It subsequently has been reported from most areas of the world where poultry is produced (7, 13, 19, 20, 38, 42, 44, 48, 51, 71). *Clostridium difficile* was isolated from a case of necrotic enteritis in a young ostrich (50).

ETIOLOGY. The cause of NE is *C. perfringens* types A (3, 9, 14, 41, 45, 52, 60, 70, 73) or C (22, 41, 51, 56, 60, 62). Some isolates of *C. perfringens* from cases of NE do not yield enough toxin in vitro to permit typing (41, 45). Alpha toxin produced by *C. perfringens* types A and C, and beta toxin produced by *C. perfringens* type C, are those believed responsible for intestinal mucosal necrosis, the characteristic lesion of NE. Both have been detected in feces of chickens with NE (41). Alpha toxin, obtained from broth culture supernatant fluids of type-A *C. perfringens*,(5, 52) is capable of producing characteristic intestinal lesions in conventional (5) or germ-free chickens (29).

C. perfringens can be readily isolated on blood agar plates incubated anaerobically at 37 C overnight. *C. perfringens* colonies on blood agar (with rabbit, human, or sheep blood) are surrounded by an inner zone of complete hemolysis and an outer zone of discoloration and incomplete hemolysis and are composed of short to intermediate, gram-positive rods without spores. Positive identification of the organism is made by inoculation of differential media (1). Most strains ferment glucose, maltose, lactose, and sucrose, do not ferment mannitol, and variably ferment salicin. Principal products of fermentation are acetic and butyric acids. Gelatin is hydrolyzed, milk is digested, and there is no indole production. Growth on egg yolk agar demonstrates presence of lecithinase and absence of lipase production. Subculturing on egg yolk agar plates, one-half of which have been spread with *C. perfringens* antitoxin, and incubating anaerobically overnight, will produce a zone of precipitation around colonies on control sides of the plate and little or no precipitation on sides spread with antitoxin (1).

PATHOGENESIS AND EPIZOOTIOLOGY. Naturally occurring outbreaks of NE have been reported in chickens from 2 wk to 6 mo of age. A majority of reports of NE have been in 2- to 5-wk-old broiler chickens raised on litter (7, 13, 31, 36, 38, 44, 48, 51, 71). However, outbreaks in 3- to 6-mo-old commercial layers raised in floor pens have also been reported (19, 42), and outbreaks of NE and coccidiosis have been reported in 12- to 16-wk-old cage-reared layer replacement pullets (18, 28). Subclinical NE in broiler chickens was significantly correlated with decreased growth rate and feed utilization, and occurred more frequently in flocks receiving high-barley diets (39).

Necrotic enteritis has been reported in turkey poults (25), 7-to 12-wk-old turkeys (32), and turkeys with concurrent ascarid infection (53) or coccidiosis (24).

C. perfringens can be found in feces, soil, dust, contaminated feed and litter, or intestinal contents (41, 43). In various outbreaks of NE, contaminated feed (20, 28, 73) and contaminated litter (72) have been incriminated as sources of infection.

Reports vary on the numbers of *C. perfringens* that can be consistently isolated from intestinal tracts of normal chickens. Some studies have found *C. perfringens* to be the principal obligate anaerobic bacterium in the intestinal tract of chickens (37, 63), whereas others have reported it only sporadically and in low numbers from small intestine of normal chickens ranging in age from recently hatched to 5 mo of age (11, 12, 61, 64, 69). Manipulating the diet can affect the population of *C. perfringens* in the intestinal tract (65), suggesting that *C. perfringens* numbers within the intestinal tract, and onset of intestinal clostridial disease in chickens, may be precipitated by the nature of the ration (51, 59). High levels of fishmeal (38, 70) or high levels of wheat (17) or barley (39) in the diet can predispose to and/or exacerbate outbreaks of NE.

Damage to the intestinal mucosa is another predisposing factor for NE (5, 70). Factors such as high-fiber litter (70) or various strains of coccidia (2, 6, 8, 9, 10, 36, 62) combined with higher than normal numbers of *C. perfringens* can result in NE. Necrotic enteritis has been experimentally reproduced in chickens (9, 21, 33, 34, 35, 57, 58), turkeys (26), and Japanese quail (22). In conventional chickens, the incidence can be from 1.3–37.3% and as high as 62.0% in specific-pathogen-free chicks (9). Necrotic enteritis can be reproduced by rearing chickens on litter in facilities where the disease has previously occurred (34, 35, 47, 72); feeding feed contaminated with *C. perfringens* (45, 70); administering vegetative cultures of *C. perfringens* intravenously (16), orally (16), or into the crop (9); administering intraduodenally

broth cultures of *C. perfringens* (3), bacteria-free crude toxins of *C. perfringens* (4), or a combination of *C. perfringens* and its toxins (5, 10); or by dosing chickens with sporulated oocysts of *Eimeria* spp. and feeding vegetative cultures of *C. perfringens* or *C. perfringens*-contaminated feed (2, 8, 9, 10).

SIGNS AND LESIONS. Clinical signs in naturally occurring outbreaks include marked to severe depression, decreased appetite, reluctance to move, diarrhea, and ruffled feathers (7, 15, 36, 44, 51, 54, 71). Clinical illness is very short; often birds are just found acutely dead.

Gross lesions in naturally occurring outbreaks are usually confined to the small intestine, primarily jejunum and ileum (Fig. 12.4A,C) (7, 15, 36, 51, 71); however, cecal lesions have been described (46). Intestines are often friable and distended with gas. The mucosa is lined by a loosely to tightly adherent yellow or green pseudomembrane. Flecks of blood have been reported, but hemorrhage is not a prominent feature. Experimentally, gross lesions characterized by a gray, thickened mucosa in the duodenum and jejunum may be observed as early as 3 hr following inoculation of *C. perfringens* (3). By 5 hr, there is necrosis of the intestinal mucosa, which progresses over time to a severe fibrinonecrotic enteritis with formation of a diphtheritic membrane (9, 62). Swollen livers with necrotic foci may accompany *C. perfringens* infections (25).

Microscopic changes in natural outbreaks are characterized primarily by severe necrosis of the intestinal mucosa with an abundance of fibrin admixed with cellular debris adherent to the necrotic mucosa (Figs. 12.4B,D) (15, 36, 46, 51, 71). Initial lesions develop at the apices of villi, and are characterized by sloughing of epithelium and colonization of the exposed lamina propria with bacilli, accompanied by coagulation necrosis. Areas of necrosis are surrounded by heterophils. Progression of lesions usually occurs from villi apices to crypts. Necrosis may extend into the submucosa and muscular layers of the intestine. Numerous large bacilli are often observed attached to cellular debris. In birds that survive, regenerative changes consist of crypt epithelial cell proliferation with a corresponding increase in mitotic figures. Epithelial cells are primarily cuboidal, with a relative decrease in goblet and columnar epithelial cells. Villi are relatively short and flat. In many outbreaks, various sexual and asexual stages of coccidia are also found in the intestine (36, 46, 51).

Microscopic changes after experimental inoculation of *C. perfringens* (3) occur as early as 1 hr following challenge, and consist of slight edema and dilation of vessels in the lamina propria, sloughed epithelial cells in the intestinal lumen, and occasional heterophils and mononuclear cells in the lamina propria. By 3 hr, marked edema, resulting in detachment of the epithelial cell layer from the lamina propria, mostly at the apex of villi, has occurred. Mononuclear cell infiltration of the lamina propria is more marked than earlier. At 5 hr, there is marked coagulation necrosis of the epithelial cell layer and lamina propria at villous tips, resulting in villus shortening. Colonization of organisms may be prominent on necrotic tissues and apices of exposed lamina propria. Blood vessels are very congested, occasionally occluded by hyaline thrombi. By 8–12 hr, there is massive necrosis of villi, in some instances reaching to the crypts, characterized by areas of amorphous eosinophilic-staining material and cell nuclei. Fibrin and cellular debris are present in the lumen.

DIAGNOSIS. Diagnosis of NE can be made based on typical gross and microscopic lesions and isolation of the causative agent. In field cases of NE, *C. perfringens* can be readily isolated from intestinal contents, scrapings of intestinal wall, or hemorrhagic lymphoid nodules by anaerobic incubation overnight at 37 C on blood agar plates (27). Identification of *C. perfringens* can be done as described under Etiology.

Diseases that must be differentiated from NE are ulcerative enteritis (UE) and *Eimeria brunetti* infection. Ulcerative enteritis is caused by *C. colinum*

12.4. Necrotic enteritis (*A–D*). Gangrenous dermatitis (*E–H*). *A.* Necrotic enteritis in a 7-wk-old broiler breeder chicken with concurrent coccidiosis. Note the hyperemia and diffuse necrosis of the mucosa with multifocal ulceration. (Munger) *B.* Intestine of a turkey showing uniform diffuse coagulation necrosis of mucosa. Deeper viable mucosal tissue is demarcated from necrotic luminal mucosal tissue by a zone of intense hyperemia, hemorrhage, and inflammation. ×20. (Barnes) *C.* Necrotic enteritis in a 6-wk-old ostrich. *C. difficile* isolated. (Munger) *D.* Histologic lesions of the case in (*C*). Severe diffuse coagulation necrosis with separation from underlying viable tissue by an intense zone of inflammation. Note numerous large gram-positive bacilli primarily located at the interface of the necrotic and viable tissue. ×30 (Munger, Barnes) *E.* Gangrenous dermatitis affecting wing of a 12-day-old broiler. Spontaneous separation of epidermis revealing edematous, hypercmic, acutely inflamed dermis. (Munger) *F.* Broiler, 6-wk-old, with gangrenous dermatitis. Extensive discolored patches of necrotic skin are present on the abdomen. (Barnes) *G.* Same bird as in (*F*). Skin reflected to show discolored muscle and serosanguinous fluid expanding underlying dermis. (Barnes) *H.* Skin from a turkey with gangrenous dermatitis. Dermis beneath a normal epidermis is markedly expanded by fluid and gas. Cutaneous muscle is undergoing rhadomyolysis. Cellular changes are minimal to absent. ×13. (Barnes)

(see section on Ulcerative Enteritis in this chapter); characteristic gross lesions are multiple areas of necrosis and ulceration in the distal small intestine and ceca and areas of necrosis in the liver. As described previously, lesions of NE are usually confined to jejunum and ileum with little or no involvement of ceca or liver. These distinguishing characteristics should allow differentiation of NE and UE. Isolation and identification of the causative agent will confirm the diagnosis. *E. bruneti* infection (see Coccidiosis, Chapter 34) causes gross lesions similar to those produced by *C. perfringens*; however, microscopic examination of fecal smears, impressions, or intestinal sections should demonstrate the presence or absence of coccidia. Finally, NE and coccidiosis often occur simultaneously in a flock, and demonstration of one or both agents is warranted.

TREATMENT AND PREVENTION. Experimentally, in vivo, a number of antibiotics placed in the feed reduce the numbers of *C. perfringens* shed in feces (66, 67, 68). These include virginiamycin, nitrovin, tylosin, penicillin, ampicillin, bacitracin, furazolidone, and efrotomycin.

Outbreaks of NE can be effectively treated by administration of lincomycin (34, 35), bacitracin (58), oxytetracycline (7), penicillin (42, 45), or tylosin tartrate (42) in the water. Bacitracin (57, 72), lincomycin (47), virginiamycin (23, 33), penicillin (51), avoparcin (39, 49, 57), and nitrovin (49) have been shown to be effective in preventing and controlling NE when placed in the feed.

Removal of fishmeal from the ration can help prevent clostridial infections in poultry (20). Probiotics such as *Lactobacillus acidophilus* and *Streptococcus faecium* reduce the severity of NE (30). Addition of *S. faecium* to cultures of *C. perfringens* results in a wide zone of inhibition (40).

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34 Protozoa

INTRODUCTION

Larry R. McDougald

Protozoa are common in poultry and other birds, and some cause moderate or severe disease. Parasitic diseases differ from viral and bacterial diseases by 1) the presence of a complicated life cycle, 2) the methods of transmission, 3) the lack of useful serologic methods for diagnosis, and 4) the means of control. Disinfection and quarantine have been of little use in control of the diseases, and control programs emphasize chemotherapy or chemoprevention rather than immunization. Prevention of coccidiosis with anticoccidial drugs administered through the feed allowed better uniformity of treatment and centralized decisions on the use of drugs. This system has proved more reliable than on-farm control and is practiced universally.

Emphasis of confinement rearing and high-density flocks have increased the infection pressure from diseases that have short, direct life cycles. In contrast, parasitic diseases that depend on an intermediate host for transmission have been practically eliminated.

Rational and effective control of all parasitic diseases depends upon accurate diagnosis of the parasite and also on the extent of the infection. Diagnosis usually depends on gross and microscopic examination of birds taken from a flock for necropsy, or on microscopic examination of feces of live birds.

Protozoa were historically placed in a single phylum, containing all one-celled animals. The complex organization and vastly different structure of protozoa led to the separation of various classes into seven different phyla (1). Two of these phyla contain species that are important parasites of poultry. The phylum Apicomplexa is characterized by the presence of an apical complex in sporozoites, and all are essentially intracellular parasites. Parasitic genera in this phylum include *Eimeria, Isospora, Haemoproteus, Leucocytozoon, Plasmodium, Toxoplasma, Sarcocystis, Wenyonella, Tyzzeria,* and *Cryptosporidium.*

The second phylum, Sarcomastigophora, includes the flagellates and amebas. Generally, they possess pseudopodia or flagella or both as locomotor organelles. Genera in this phylum that are important to poultry include *Histomonas, Trypanosoma, Chilomastix, Entamoeba, Endolimax,* and *Hexamita.*

Encephalitozoon cuniculi, a protozoan in a third phylum, Microspora, recently has been discovered infecting chickens. The protozoan is egg-transmitted and infection can be associated with embryo mortality but is usually inapparent. Affected birds may show inactivity, lameness, mild diarrhea, and weight loss. Parasites have been identified in the digestive tract, urogenital organs, and muscle. In embryos, brain and heart also were found to be infected (2, 3).

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COCCIDIOSIS

Larry R. McDougald and W. Malcolm Reid

INTRODUCTION. Coccidiosis is a disease of almost universal importance in poultry production. The protozoan parasites of the genus *Eimeria* multiply in the intestinal tract and cause tissue damage, with resulting interruption of feeding and digestive processes or nutrient absorption; dehydration;

blood loss; and increased susceptibility to other disease agents. Historically, the spectacular onset of coccidiosis with bloody diarrhea and high mortality inspired awe and dread on the part of poultry growers and fanciers. Like many parasitic diseases, coccidiosis is largely a disease of young animals because immunity quickly develops after exposure and gives protection against later disease outbreaks. Unfortunately, there is no cross-immunity between species of *Eimeria* in birds, and later outbreaks may be the result of different species. The short, direct life cycle and high reproductive potential of coccidia in poultry intensifies the potential for severe outbreaks of disease in the modern poultry house, where 15–30,000 chickens may be reared in total confinement.

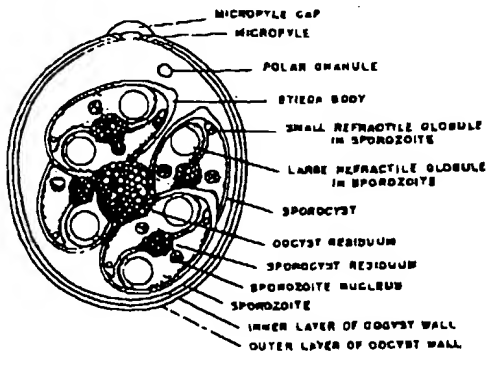
Coccidiosis may strike any type of poultry in any type of facility. The disease may be mild, resulting from ingestion of a few oocysts, and may escape notice, or may be severe as a result of ingestion of millions of oocysts. Most infections are relatively mild, but because of the potential for the disastrous outbreak and the resulting financial loss, almost all young poultry are given continuous medication with low levels of anticoccidial drugs, which prevent the infection or reduce infections to a low, immunizing level. Immunity is not as important in broiler chickens, which may be kept only for 6–8 wk before market, as in layers, turkeys, and breeder birds, which may be kept much longer. Vaccines against coccidiosis have met with limited success, and have been used mostly in breeder pullets and in turkeys. Vaccination of broilers has rarely been practiced because even light infections with some species of coccidia can affect weight gain, feed conversion, and pigmentation of the skin.

CLASSIFICATION AND TAXONOMIC RELATIONSHIPS. The biology and taxonomy of coccidia were reviewed by Long (18) and Pellerdy (25). Although several genera of coccidia are known to infect some types of birds, those most often encountered in poultry belong to the genus *Eimeria* described in this section or the genus *Cryptosporidium* discussed in another section of this chapter. Species of *Eimeria* are frequently described from the morphology of the oocyst, a thick-walled zygote shed in fecal matter by the infected host. Oocysts are enclosed in a thick outer shell and consist of a single cell that begins the process of sporulation to yield the infective stage in about 48 hr. Infective oocysts contain four sporocysts, which in turn contain two sporozoites (Fig. 34.1).

The closely related parasites *Sarcocystis* and *Toxoplasma*, as well as avian malaria, are discussed in the subchapter Other Blood and Tissue Protozoa.

When oocysts are ingested, the oocyst wall is crushed in the gizzard, and the sporozoites are released from sporocysts by the action of chymotrypsin and bile salts in the small intestine. Sporozoites enter epithelial cells or are taken into intraepithelial lymphocytes, where development may begin. Species of coccidia are identified on the basis of: 1) oocyst morphology, 2) host specificity, 3) immune specificity, 4) appearance and location of gross lesions within the natural host, and 5) length of the prepatent period. The host specificity of *Eimeria* in birds and mammals is very strict, so that parasites from different species of birds or animals can be considered different species even though they may have similar-appearing oocysts.

Life Cycle. Coccidiosis differs from bacterial and viral diseases in the self-limiting nature of its development. The life cycle of *E. tenella* (Fig. 34.2) is typical of all *Eimeria*, although some species vary in the number of asexual generations and the time required for each developmental stage. After the oocyst wall is crushed in the gizzard and the sporozoites are released, the sporozoites enter cells in the mucosa of the intestine and begin the cell cycle leading to reproduction. At least two generations of asexual development, called schizogony or merogony, lead to a sexual phase, where small, motile microgametes seek out and unite with macrogametes. The resulting zygote matures into an oocyst, which is released from the intestinal mucosa and is shed in the feces. With each species, the reproductive potential from a single ingested oocyst is fairly constant. The entire process takes 4-6 days, depending on species, although oocysts may be shed for several days after patency is reached. In some species (*E. tenella, E. necatrix*), the maximum tissue damage may occur when second-generation schizonts rupture to release merozoites. Other species may have small schizonts, which cause lit-



34.1. Diagram of sporulated oocyst of genus *Eimeria*.

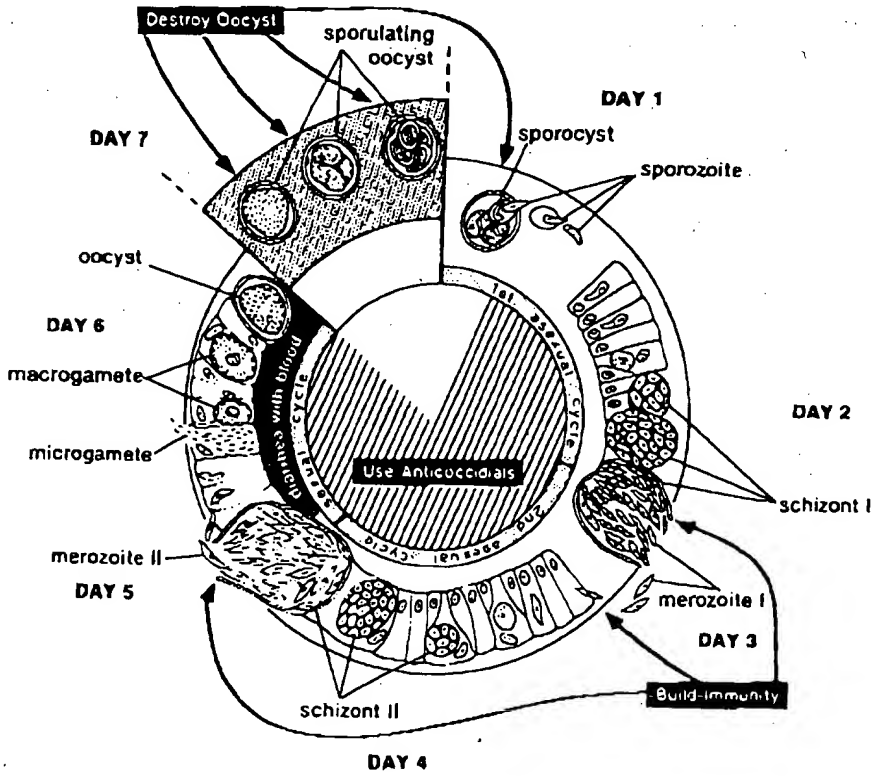
tle damage, but the gametocytes may elicit a strong reaction with cellular infiltration and thickened, inflamed tissues.

Relationship between Coccidiosis and Other Poultry Diseases. The tissue damage and changes in intestinal tract function may allow colonization by various harmful bacteria, such as *Clostridium perfringens*, leading to necrotic enteritis (12, 19), or *Salmonella typhimurium* (2, 3).

Immunosuppressive diseases may act in concert with coccidiosis to produce a more severe disease. Marek's disease may interfere with development of immunity to coccidiosis (4), and infectious bursal disease (IBD) may exacerbate coccidiosis, placing a heavier burden on anticoccidial drugs (21).

COCCIDIOSIS IN CHICKENS. Coccidiosis remains one of the most expensive and common diseases of poultry production in spite of advances in chemotherapy, management, nutrition and genetics. The disease is often diagnosed in birds brought to diagnostic laboratories (1), but the vast majority of cases are diagnosed in the field, and handled by poultry service personnel. The current expense for preventive medication exceeds $90 million in the USA and over $300 million worldwide.

Incidence and Distribution. Coccidia are almost universally found wherever chickens are raised. Their strict host specificity eliminates wild birds as sources of infection. The most common means of spread of coccidia is mechanical, by personnel who move between pens, houses, or farms. Coccidial infections are self-limiting and depend largely on the number of oocysts ingested and on the immune status of the bird. Surveys in North and South America revealed coccidia present in almost all broiler farms (22, 23). Very high percentages of positive flocks were also reported from Europe (5, 17). Oocysts in the litter or droppings of broiler chickens are usually most numerous at 4–5 wk of age, and generally decline thereafter. Few oocysts are found after birds are removed from a farm, be-



34.2. The 7-day life cycle of *E. tenella* includes two or more asexual and one sexual cycle during the 6 days after an oocyst has been swallowed by the host. The new generation of oocysts must sporulate (day 7) after being passed by the host before becoming infective.

cause poultry litter or droppings are poor environments for their survival. The ubiquitous nature of poultry coccidia precludes the possibility of elimination or prevention of coccidia by quarantine, disinfection, and sanitation.

Etiology. Nine species of *Eimeria* have been described from chickens (Table 34.1), but some are questionable. Similarly, several species of *Cryptosporidium* were described, but possibly only one or two names are valid owing to the lack of species specificity. *Cryptosporidium* spp. are often diagnosed, but clinical disease is not often seen (9). Concurrent infection with two or more species of coccidia is common.

Characteristics useful in identification of species are as follows: 1) location of the lesions in the intestine, 2) appearance of the gross lesion, 3) oocyst size, shape, and color, 4) size of schizonts and merozoites, 5) location of parasites in tissues (type of cell parasitized), 6) minimum prepatent period in experimental infections, 7) minimum time for sporulation, 8) immunogenicity against pure strains. In recent years, more emphasis has been placed on biochemical and physiologic identification of coccidia. A promising new tool for species identification is electrophoresis of metabolic enzymes (28). For diagnostic purposes, the traditional characteristics are adequate, and a satisfactory diagnosis can be made from Table 34.2. Cross-immunity and biochemical studies require pure species isolates propagated from single oocysts. Monoclonal antibodies are useful in serologic diagnosis, but have not been suitably specific to distinguish species, probably because of common antigens. The severity of infection is often graded on a scale of 0–4 as described by Johnson and Reid (16), where 0 = normal and 4 = maximum lesion.

***EIMERIA ACERVULINA* TYZZER 1929.** This species is the most frequently encountered in commercial poultry in North and South America. Oocysts are ovoid and often show thinning of the shell at the small end. The average size of oocysts is 18.3 × 14.6 μm, but the range is 17.7–20.2 × 13.7–16.3 μm.

PATHOGENICITY. Severity of infection may vary with the isolate, the number of oocysts ingested and the immune state of the bird. Ingestion of 1000, 30,000, 100,000 or 1,000,000 oocysts by young White Rock chicks resulted in mild to severe coccidiosis, with lesion scores ranging from 1+ (1000 oocysts) to 4+ (1,000,000 oocysts) (26). Reduction in rate of weight gain was also proportional to the infective dose. Heavy infections often cause lesions to coalesce, and sometimes mortality may result. Light to moderate infections may produce little effect on weight gain and feed conversion, but may cause loss of carotenoid and xanthophyll pigments from the blood and skin because of reduced absorption in the small intestine. The intestinal mucosa may be thickened, resulting in poor feed conversion. Egg production may be reduced in laying birds.

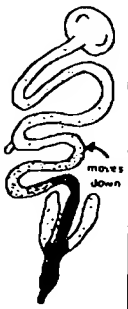
GROSS LESIONS AND HISTOPATHOLOGY. Lesions can often be seen from the serosal surface of the small intestine. The intestinal mucosa may at first be thin and covered with white plaques, which tend to arrange in transverse fashion and cause a ladderlike appearance because of the striations. The intestine may be pale and contain watery fluid. The gross lesion in light infections is limited to the duodenal loop, with only a few plaques/cm, but in heavy infections lesions may extend some distance through the small intestine, and plaques may overlap or coalesce; they are generally smaller in heavy infections due to crowding. The lesions are comprised of schizonts, gametocytes, and developing oocysts. Microscopy of smears from intestinal lesions usually reveals numerous oocysts.

Histopathology of the small intestine reveals the ovoid gametocytes lining the mucosal cells on the villi. In moderate to heavy infections, the tips of villi are broken off, leading to truncation and fusion of villi and thickening of the mucosa. Some cells may contain more than one parasite. Schiff's reagent will stain the macrogametes and developing oocysts a brilliant red, because of the polysaccharide used in oocyst wall formation.

***EIMERIA BRUNETTI* LEVINE 1942.** About 10–20% of field isolates in recent surveys in the United States and South America contained *E. brunetti* (22). The oocysts of *E. brunetti* average 24.6 × 18.8 μm, and are easily confused with *E. tenella*. This species is found in the lower small intestine, usually from the yolk sac diverticulum to near the cecal juncture. In severe cases, the lesion may extend from the gizzard to the cloaca and extend into the ceca (Fig. 34.3 E–H). Most field infections are difficult to recognize based on gross lesions and can be confirmed only with the aid of microscopy.

PATHOGENICITY. Although less serious than *E. tenella* or *E. necatrix*, *E. brunetti* is capable of producing moderate mortality, loss of weight gain, poor feed conversion and other complications. Inoculation with 100,000–200,000 oocysts will frequently cause 10–30% mortality and reduced gain in survivors. Light infections of *E. brunetti* are easily overlooked unless careful attention is paid to the lower small intestine. Such infections can cause reduced weight gain and poor feed conversion even though gross lesions are not clearly apparent.

Table 34.1. DIFFERENTIAL CHARACTERISTICS FOR 9 SPECIES OF CHICKEN COCCIDIA ⊛

	CHARACTERISTICS	E. acervulina	E. brunetti	E. maxima	E. mitis !	E. mivati I	E. necatrix	E. praecox	E. tenella	SPECIES OF DOUBTFUL VALIDITY E. hagani
DIAGNOSTIC CHARACTERISTICS IN RED	ZONE									
MACROSCOPIC LESIONS	PARASITIZED									
	MACROSCOPIC LESIONS	light infection: whitish foci lesions sometimes in ladder-like streaks heavy infection: plaques coalescing thickened intestinal wall	coagulation necrosis mucoid bloody enteritis in cranial intestine	thickened walls, mucoid, blood - tinged exudate, petechiae	no discrete lesions in intestine, mucoid exudate	light infection rounded plaques of oocysts heavy infection thickened walls coalescing plaques	ballooning white spots (schizonts) petechiae, mucoid blood - tinged exudate	no lesions, mucoid exudate	cecal hemorrhage and tumefaction, thickening, whitish mucosa, occasional clotted blood	pinhead hemorrhages petechiae
MICROSCOPIC CHARACTERISTICS	MILLIMICRONS OOCYSTS REDRAWN FROM ORIGINALS	10 20 30	10 20 30	10 20 30	10 20 30	10 20 30	10 20 30	10 20 30	10 20 30	not available
	LENGTH x WIDTH μ LENGTH = WIDTH =	AV = 18.3 x 11.6 17.7 - 20.2 13.7 - 16.3	24.6 x 18.8 20.7 - 30.3 18.1 - 24.2	30.5 x 20.7 21.5 - 42.5 16.5 - 29.8	15.6 x 14.2 11.7 - 18.7 11.0 - 19.0	15.6 x 13.4 11.4 - 19.9 10.5 - 16.2	20.1 x 17.2 13.2 - 22.7 11.3 - 18.3	21.3 x 17.1 19.8 - 21.7 15.7 - 19.8	22.0 x 19.0 19.5 - 26.0 16.5 - 22.8	18.1 x 17.5 15.8 - 20.9 14.3 - 19.5
	OOCYST SHAPE AND INDEX LENGTH/WIDTH	ovoid 1.25	ovoid 1.31	ovoid 1.47	subspherical 1.09	ellipsoid to broadly ovoid 1.16	oblong ovoid 1.19	ovoidal 1.24	ovoid 1.16	broadly ovoid 1.08
	SCHIZONT, MAX IN MICRONS	10.5	30.0	9.4	15.1	17.3	55.9	20	51.0	
	PARASITE LOCATION IN TISSUE SECTIONS	epithelial	2nd generation schizonts subepithelial	gamaocytes subepithelial	apical	epithelial	2nd generation schizonts subepithelial	epithelial	2nd generation schizonts subepithelial	epithelial
LIFE HISTORY CHARACTERISTICS	MINIMUM PREPATENT PERIOD HR	97	120	121	93	95	139	83	115	99
	SPORULATION TIME MINIMUM (HR)	17	18	20	15	12	18	12	18	18

1 = From Norton and Joyner (1980)
1 = As described by Edgar and Siebold (1964)
⊛ = Compiled from various sources (1982)

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Table 34.2. Anticoccidial drugs for treatment of coccidiosis in chickens

Trade or Empirical Name (Manufacturer)	Feed or Water	Active Ingredient, Treatment Duration	First Approval by FDA	Drug Withdrawal (Days before Slaughter)
Sulfamethazine (American Cyanamid)	Water	0.1%: 2 days; 0.05%: 4 days	1947	10
SQ,[a] sulfaquinoxaline (Merck); Sulquin[a] (Salsbury)	Feed	0.1%: 2-3 days on, 3 off followed by 0.05%: 2 on, 3 off, 2 on	1948	10
Amprol,[a] amprolium (Merck)	Water	0.012-0.024%: 3-5 days; 0.006%: 1-2 weeks	1960	0
Esb$_3$,[a] sodium sulfachloropyrazine monohydrate (Squibb)	Water	0.03%: 3 days	1967	4
Agribon,[a] Albon,[a] sulfadimethoxine (Hoffmann-La Roche)	Water	0.05%: 6 days	1968	5

FDA, Food and Drug Administration.
[a]Registered trade name.

GROSS LESIONS AND HISTOPATHOLOGY. At early stages of infection, the mucosa of the lower small intestine may be covered with tiny petechiae and have some thickening and loss of color. In heavy infections, the mucosa is badly damaged, with coagulation necrosis appearing on days 5–7 post infection (PI) and with a caseous eroded surface over the entire mucosa. The coagulated blood and mucosa will be apparent in the droppings. Thickening of the mucosa and edematous swelling occurs in severe infections, especially on the 6th day PI.

The asexual stages of first- and second-generation schizogony generally occur in the upper small intestine. Histopathology on the 4th day of infection reveals schizonts, cellular infiltration, and some damage to the mucosa. By the 5th day, many of the tips of villi are broken off. Merozoites invade the epithelium and develop into sexual stages in the lower small intestine and ceca. In severe cases, the villi may be completely denuded, and in some instances only the basement membranes remain.

***EIMERIA HAGANI* LEVINE 1938.** The taxonomic status of *E. hagani* is in doubt because the original description was incomplete. This species reportedly produced hemorrhagic spots, catarrhal inflammation, and watery intestinal contents and was moderately pathogenic. Unless research is forthcoming to establish the characteristics of this species and existence in field infections, it will likely be declared invalid.

***EIMERIA MAXIMA* TYZZER 1929.** The mid-small intestine is often parasitized with *E. maxima*, from below the duodenal loop past the yolk sac diverticulum, but in heavy infections the lesions may extend throughout the small intestine. *E. maxima* is an easy species to recognize because of the characteristic large oocysts, 30.5 × 20.7 μm (21.5–42.5 × 16.5–29.8), which usually have a distinctive yellowish color (Fig. 34.4A,F,G,H,I,J). There is often an abundance of yellow-orange mucus and fluid in the midgut. This species can be differentiated from *E. necatrix* by the lack of large schizonts associated with the lesions, and from *E. brunetti* by the larger oocysts and the appearance of the lesions.

PATHOGENICITY. This species is moderately pathogenic. Infection with 200,000 oocysts is usually sufficient to cause poor weight gain, morbidity, diarrhea, and sometimes mortality. There is often extreme emaciation, pallor, roughening of feathers, and inappetence. Producers interested in maintaining good skin color in chickens must be concerned with subclinical infections because of the effect of this species on absorption of the xanthophyll and carotenoid pigments in the small intestine.

GROSS LESIONS AND HISTOPATHOLOGY. Minimal tissue damage occurs with the first two asexual cycles, which develop superficially in the epithelial cells of the mucosa. When the sexual stages develop in deeper tissues on the 5th to 8th days PI, lesions develop because of congestion and edema, cellular infiltration, and thickening of the mucosa. Infected host cells become enlarged, pushing into the subepithelial zone. Microscopic hemorrhages occur near the tips of the villi, and foci of infection can be seen from the serosal surface. The intestine may be flaccid and filled with fluid, and the lumen

often contains yellow or orange mucus and blood. This condition has been described as "ballooning." Microscopic pathology is characterized by edema and cellular infiltration, developing schizonts through day 4, and sexual stages (macrogametes and microgametes) in deeper tissues on days 5–8. In severe infections, there is considerable disruption of the mucosa.

***EIMERIA MITIS* TYZZER 1929.** The lower small intestine is the normal site of this parasite, from the yolk sac diverticulum to the cecal necks. The lesions are normally indistinct with this species, but the potential for pathogenic effects on weight gain and morbidity was recently demonstrated.

PATHOGENICITY. Infection with 1–1.5 million oocysts will reduce weight gain and cause morbidity and loss of pigmentation. The lack of distinct gross lesions causes this species to be overlooked or misdiagnosed in subclinical infections.

GROSS LESIONS AND HISTOPATHOLOGY. Clinically, the gross lesion is very slight and can be easily overlooked. The lower small intestine appears pale and flaccid, and microscopic examination of smears from the mucosal surface may reveal numerous tiny oocysts (15.6 × 14.2 μm). The infection is easily distinguished from *E. brunetti* by the smaller, round oocysts. In light infections, the appearance of the gross lesion may be similar to *E. brunetti*. The gross lesion of this species is unremarkable because the developing parasites do not tend to localize in colonies as do other species, and the schizonts and gametocytes are superficial in the mucosa.

***EIMERIA MIVATI* EDGAR AND SIEBOLD 1964.** This parasite was first identified as a small strain of *E. acervulina* (7). The parasitized zone reportedly extends from the duodenal loop to the ceca and cloaca. Early lesions appear in the duodenum and later in the midgut and lower small intestine. In light infections, isolated lesions resemble those of *E. acervulina*, but are more circular in shape. These lesions, representing colonies of gametocytes and developing oocysts, may be seen from the serosal surface of the gut. Infection with 1,000,000 oocysts of *E. mivati* causes reduced weight gain and morbidity. Occasional mortality occurs in experimental infections.

Recent work with isoenzymes has caused some workers to question the validity of *E. mivati*. Examination of laboratory cultures has failed to produce a bona fide culture of *E. mivati*, but there have been no extensive field studies aimed at settling this controversy. While there is no convincing evidence for the existence of this species, not all field observations can be easily explained within the taxonomic limits of other described species. Further work will be needed to settle the taxonomic status of this species.

***EIMERIA NECATRIX* JOHNSON 1930.** Because of the spectacular lesions in the small intestine, this species was one of the best known by early poultry producers. The lesion is found in the small intestine in approximately the same location as *E. maxima* (Fig. 34.3 A-D). Probably because of the low reproductive capability of *E. necatrix*, it is not able to compete with other coccidia and is diagnosed mostly in older birds such as brooder pullets or layer pullets 9–14 wk old. The intestine is often dilated to twice its normal size (ballooning) and the lumen may be filled with blood. The oocysts are near in size to those of *E. tenella* and are found only in the ceca. The sexual stages do not develop in the intestine where the lesions are found, but in the ceca where they compete for space with *E. tenella*. The developing gametocytes are scattered and not found in colonies.

PATHOGENICITY, GROSS LESIONS, AND HISTOPATHOLOGY. Some gross lesions may be associated with first-generation schizogony at 2–3 days PI. By the 4th day PI, the intestine may be ballooned, the mucosa thickened, and the lumen filled with fluid, blood, and tissue debris. From the serosal surface, the foci of infection can be seen as small white plaques or red petechiae. Smears examined microscopically on the 4–5th days may contain numerous clusters of large (66 μm) schizonts, often containing hundreds of merozoites. The clusters of schizonts deep in the mucosa often penetrate the submucosa and damage the layers of smooth muscle and destroy blood vessels. In these instances, the foci are large enough to be seen from the serosal surface. Later, scar tissue may be seen where epithelial regeneration is incomplete. Few pathogenic effects are seen with the invasion of the cecal mucosa by the third-generation schizonts and gametocytes because of the scattered, noncolonizing nature of these stages. The third-generation schizonts produce only 6–16 merozoites, compared with the hundreds of merozoites produced by the second-generation schizonts in the small intestine.

Lesions may extend throughout the small intestine in severe infections, causing dilation (ballooning) and thickening of the mucosa. The lumen may be filled with blood and pieces of mucosal tissue. From the serosal surface, the infection may be seen as white or red foci, or in dead birds the foci will be white and black, giving the appearance of "salt and pepper." Microscopic examination of smears from the mucosal surface reveals numerous clusters of large schizonts, which are characteristic for this

species and distinguishes it from others that overlap in habitat. Also, oocysts are never associated with lesions of this species.

Infection with 75,000–100,000 oocysts is sufficient to cause severe weight loss, morbidity, and mortality. Survivors may be emaciated, suffer secondary infections, and lose pigmentation. Droppings of infected birds often contain blood, fluid, and mucus. This species and *E. tenella* are the most pathogenic of the chicken coccidia. Naturally occurring infections have caused mortality in excess of 25% in commercial flocks, and in experimental infections 100% mortality is possible.

***EIMERIA PRAECOX* JOHNSON 1930.** This species is named from the short prepatent period (about 83 hrs); hence a "precocious" parasite. Even though *E. praecox* is often overlooked because there are no prominent lesions, there may be reduced weight gain, loss of pigmentation, extreme loss of fluids, and poor feed conversion.

PATHOGENICITY, GROSS LESIONS, AND HISTOPATHOLOGY. The gross lesion consists of watery intestinal contents and sometimes mucus and mucoid casts. Most of the infection is confined to the duodenal loop. Small pinpoint hemorrhages may be seen on the mucosal surface on the 4th and 5th days of infection. Recent studies suggest that this species may cause morbidity and reduced weight gain (10). Dehydration may result from the extreme fluid loss caused by severe infections. The epithelial cells of the sides of the villi (but not the tips) are most often infected. There may be several parasites in each cell. Three to four asexual generations are followed by the sexual stages. The oocysts are generally larger than those of other species found in the duodenum. At 21.3 × 17.1 μm, they are larger than *E. acervulina*, *E. mivati*, and *E. mitis* and smaller than *E. maxima*. Little tissue reaction has been described.

***EIMERIA TENELLA* (RAILLIET AND LUCET 1891) FANTHAM 1909.** Coccidiosis caused by *E. tenella* is the best known of the avian types, partly because of the spectacular disease it causes, and partly because of its widespread importance in commercial broilers. This species inhabits the ceca and adjacent intestinal tissues, causing a severe disease characterized by bleeding, high morbidity and mortality, lost weight gain, emaciation, and other signs attributed to coccidiosis. Diagnosis is dependent upon finding cecal lesions with accompanying clusters of large schizonts or (later) oocysts (Fig. 34.3 I–L)

PATHOGENICITY, PATHOGENESIS, AND EPIZOOTIOLOGY. Experimental inoculation with 100,000 sporulated oocysts can cause morbidity, mortality, and greatly reduced weight gain, making this one of the most pathogenic species in chickens. Inoculation with 1000–3000 oocysts is sufficient to cause bloody droppings and other signs of infection. The most pathogenic stage is the second-generation schizont, which matures at 4 days PI. Like *E. necatrix*, this species produces colonies of large schizonts, which may contain hundreds of merozoites. The schizonts develop deep in the lamina propria, so that the mucosa is badly disrupted when the schizonts mature and merozoites are released. Onset of mortality in a flock is rapid. Most of the mortality occurs between 5 and 6 days PI, and in acute infections it may follow the first signs of infection by only a few hours. Blood loss may reduce the erythrocyte count and hematocrit value as much as 50%. The maximum effect on weight gain is seen at 7 days PI. Some of the weight lost from dehydration may be regained quickly, but growth will always lag behind that of uninfected birds. The exact cause of death is not known, but toxic factors are suspected. Blood loss alone does not account for mortality. In a few cases, death may result from gangrenous or ruptured cecal pouches. Extracts of infected cecal pouches produce acute blood coagulation and death when injected intravenously into other chicks. The possible role of bacterial products in mortality from coccidiosis is suggested by the lack of mortality from *E. tenella* in germ-free chicks.

GROSS LESIONS AND HISTOPATHOLOGY. Even during maturation of the first generation of schizonts, small foci of denuded epithelium may be seen. By the 4th day PI, the second-generation schizonts are maturing and hemorrhages are apparent. The cecal pouch may become greatly enlarged and distended with clotted blood and pieces of cecal mucosa in the lumen. On the 6th and 7th days, the cecal core becomes hardened and drier, eventually it is passed in the feces. Regeneration of the epithelium is rapid and may be complete by the 10th day. The infection can usually be seen from the serosal surface of the ceca as dark petechiae and foci, which become coalesced in more severe infections. The cecal wall is often greatly thickened because of edema and infiltration, and later scar tissue.

Microscopically, the first-generation schizonts are widely scattered and mature at 2–3 days PI. Small focal areas of hemorrhage and necrosis may appear near blood vessels of the inner circular muscles of the muscularis layer. Heterophil infiltration of the submucosa proceeds rapidly as the large second-generation schizonts develop in the lamina propria. These are found in clusters or colonies that generally are progeny of a single first-generation schizont. Maturation of the second-generation para-

sites is accompanied by excessive tissue damage, bleeding, disruption of the cecal glands, and often complete destruction of the mucosa and muscularis layer. Oocysts are seen on microscopic examination on the 6th and 7th days, when macrogametes and motile microgametes can often be seen. Regeneration of the epithelium and glands may be complete by the 10th day in light infections, but the epithelium may never completely recover in severe infections. Lost muscularis mucosa is not replaced and the submucosa becomes densely fibrosed.

Epizootiology

NATURAL AND EXPERIMENTAL HOSTS. The chicken is the only natural host of the species described above. Reports of these species of *Eimeria* infecting other birds can be considered spurious. Cross-transmission of *Eimeria* spp. from chickens to other host species has been unsuccessful except for a few instances where immunocompromised birds were used.

Chickens of all ages and breeds are susceptible to infection. Immunity develops quickly, limiting further infection. Newly hatched birds are sometimes not fully susceptible to infection because of insufficient chymotrypsin and bile salts in the intestines to cause excystation. Outbreaks are common at 3–6 wk of age and are rarely seen in poultry flocks at less than 3 wk. Surveys of coccidia in broiler houses in Georgia demonstrated the manner in which oocysts of coccidia build up during growth of a flock, then decline as the birds become immune to further infection (27). This "self-limiting" nature of coccidial infections is widely known in chickens and other poultry. There is no stimulation of cross-immunity between species of coccidia. Thus, several outbreaks of coccidiosis are possible in the same flock, with different species involved in each. Breeder pullets and layer pullets are at greatest risk because they are kept on litter for 20 wk or more. Normally the infections with *E. acervulina*, *E. tenella*, and *E. maxima* are seen at 3–6 wk of age, then *E. necatrix* at 8–18 wk of age.

Coccidiosis rarely occurs in layers and breeders because of prior exposure to coccidia and resulting immunity. In a few instances, a flock may not be exposed to a particular species, or the immunity may lapse because of other diseases. Outbreaks of any species in layers can reduce or eliminate egg production for several wk.

TRANSMISSION AND VECTORS. Ingestion of viable sporulated oocysts is the only natural method of transmission. Infected chickens may shed oocysts in the feces for several days or wk. The oocysts in feces become infective through the process of sporulation within 2 days. Susceptible birds in the same flock may ingest the oocysts through the litter-pecking activities common to chickens.

Although there are no natural intermediate hosts for the *Eimeria* spp., oocysts can be spread mechanically by many different animals, insects, contaminated equipment, wild birds, and dust. Oocysts are generally considered resistant to environmental extremes and to disinfectants, although survival time varies with conditions. Oocysts may survive for many wk in soil, but survival in poultry litter is limited to a few days because of the ammonia released by composting and the action of molds and bacteria. Viable oocysts have been reported from the dust inside and outside broiler houses, as well as from insects in poultry litter (27). The darkling beetle, common in broiler litter, is a mechanical carrier of oocysts. Transmission from one farm to another is facilitated by movement of personnel and equipment between farms and by the migration of wild birds, which may mechanically spread the oocysts. New farms may remain free of coccidia for most of the first growout of chickens until the introduction of coccidia to a completely susceptible flock. Such outbreaks, which are usually more severe than those experienced on older farms, are often called "the new-house coccidiosis syndrome."

Oocysts may survive for many wk under optimal conditions but will be quickly killed by exposure to high or low temperatures or drying. Exposure to 55 C or freezing kills oocysts very quickly. Even 37 C is fatal when continued for 2–3 days. Sporozoites and sporocysts can be frozen in liquid nitrogen with appropriate cryopreservation technique, but oocysts cannot be adequately infiltrated with cryoprotectants to effect survival. Threat of coccidiosis is less during hot dry weather and greater in cooler wetter weather.

Diagnosis. Coccidiosis can best be diagnosed from birds killed for immediate necropsy. Attempts to identify characteristic lesions in birds that have been dead for 1 hr or longer are frustrated by the postmortem changes that begin quickly in the intestine. The entire intestinal tract should be examined. A microscope should be available for use in looking for special diagnostic characteristics such as the large schizonts of *E. necatrix* or the small round oocysts of *E. mitis*. The finding of a few oocysts by microscopic examination of smears from the intestine indicates the presence of infection, but not a diagnosis of clinical coccidiosis. Coccidia are often present in the intestines of birds 3–6 wk old in most flocks. Coccidiosis should be diagnosed if the gross lesions are serious, or if other economic parameters are threatened. Diagnosis should be based on finding of lesions and confirmatory microscopic stages

on necropsy of typical birds from the flock, rather than from culls.

Microscopic Examination. Many stages of coccidia can be seen in smears taken from the suspected lesion. A small amount of mucosal scraping should be diluted with saline on a slide, then covered with a coverslip. Oocysts or macrogametes are most easily seen, but in many cases the lesion is caused by maturing schizonts. Presence of clusters of large schizonts in the midgut area is pathognomonic for *E. necatrix*, while a similar finding in the ceca indicates *E. tenella*.

Oocyst size and shape are less useful as diagnostic characteristics in chickens than once thought, because of the extensive overlapping in size of the species. Measurement of 30–50 oocysts of the predominant type of oocyst usually gives a good indication of the size of the unknown species. This information is useful in conjunction with other observations in the identification of species in field cases.

Lesion Scoring. The severity of lesions is generally proportionate to the number of oocysts ingested by the bird and correlates with other parameters such as weight loss and droppings scores. The most commonly used system was devised by Johnson and Reid (16). By this system a score of 0 to 4 is assigned to a bird where 0 = normal and 4 = most severe case. This technique is most useful in experimental infections, where the dose of oocysts and medicaments are controlled, and the species are known. In the field, lesion scoring is generally useful in gauging the severity of infections. Even though there are several species of coccidia that may be present at some time, only four separate sections of the intestine are usually scored. These are 1) the duodenum (upper), 2) the midgut from the duodenum past the yolk sac diverticulum, 3) the lower small intestine from the yolk sac diverticulum to the cecal junctures, and 4) the ceca.

Droppings Score. In laboratory infections, the droppings score may be used in the same manner as lesion score for a rapid and fairly reliable rating of the infection (24). The extent of abnormal droppings is rated on a scale of 0–4, where 4 = maximum diarrhea, with mucus, fluid, and/or blood.

Histopathology Methods. Ordinary methods in histopathology are satisfactory for routine examination of tissues infected with coccidia. Staining of sections with H & E or other common histologic stains will demonstrate developing stages. There are specialized techniques that will identify specific stages: Staining with Schiff's reagent gives a brilliant red color with the polysaccharide associated with the refractile body and with wall-forming bodies in the macrogamete. Monoclonal antibodies conjugated with fluorescent markers such as fluorescein are highly useful in research because specific stages of parts of cells can be readily identified.

Procedures Used in Species Identification. One of the oldest techniques takes advantage of the lack of cross-immunity when birds are infected with one species of coccidia. If pure cultures of coccidia are used to infect groups of birds repeatedly, they will become immune to that species. If a test culture produces patent infections in immunized birds, it must be of a different species. In this way, by process of elimination, the species can be determined. This technique is time consuming and requires extensive laboratory isolation facilities and access to pure cultures of known species of coccidia, but has proved extremely useful as a research tool. Pure species cultures of coccidia are difficult to maintain because they must be propagated in strict isolation to prevent contamination.

Preservation of Coccidia for Experimental Work. Droppings or litter collected in the field, or intestinal contents in the diagnostic lab, can be saved for isolation of coccidia in a solution of 2–4% potassium dichromate. Aeration of oocyst suspensions is necessary to allow sporulation. A good-quality aquarium pump is highly effective and can be regulated with valves and tubes to service several bottles at one time. For short-term storage, suspensions of oocysts may be refrigerated.

Prevention and Control

CONTROL OF COCCIDIOSIS BY CHEMOTHERAPY. Early emphasis in chemotherapy was centered on the treatment of outbreaks with sulfonamides or other compounds after signs of infection were apparent. Soon, the concept of preventive medication emerged with the realization that most of the damage is done once signs of coccidiosis are widespread in a flock. Today almost all broiler flocks receive preventive medication, and treatment is used as a last resort (Table 34.3). The historical aspects of chemotherapy have been reviewed extensively by McDougald (20).

CHARACTERISTICS OF ANTICOCCIDIAL DRUGS. All types of drugs used for coccidiosis control are unique in the mode of action, the way in which parasites are killed or arrested, and the effects of the drug on the growth and performance of the bird. Following are the most important characteristics.

Spectrum of Activity. There are several important

Table 34.3. Preventive anticoccidials approved by FDA for use in feed formulation

Trade or Empirical Name, Approved Level (Manufacturer)	Trade Name	First Approval by FDA	Drug Withdrawal (Days before (Slaughter)
Sulfaquinoxaline, 0.015-0.025% (Merck)	SQ, Sulquin	1948	10
Nitrofurazone, 0.0055% (Hess & Clark; Smith-Kline)	nfz, Amifur	1948	5
Arsanilic acid or sodium arsanilate, 0.04% for 8 days (Abbott)	Pro-Gen	1949	5
Butynorate, 0.0375% for turkeys (Solvay)	Tinostat	1954	28
Nicarbazin, 0.0125% (Merck)	Nicarb	1955	4
Furazolidone, 0.0055-0.011% (Hess & Clark)	nf-180	1957	5
Nitromide, 0.025% + sulfanitran, 0.03% + roxarsone, 0.005% (Solvay)	Unistat-3	1958	5
Oxytetracycline, 0.022% (Pfizer)	Terramycin	1959	3
Amprolium, 0.0125-0.025% (MSD-AGVET)	Amprol	1960	0
Chlortetracycline, 0.022% (American Cyanamid)	Aureomycin	1960	(see feeding restrictions) (higher levels, 5 days)
Zoalene, 0.004-0.0125% (Solvay)	Zoamix	1960	
Amprolium, 0.0125% + ethopabate, 0.0004/0.004% (Merck)	Amprol Plus, Amprol Hi-E	1963	0
Buquinolate, 0.00825% (Norwich-Eaton)	Bonaid	1967	0
Clopidol or meticlorpindol, 0.0125-0.025% (A. L. Laboratories)	Coyden	1968	0 days at 0.0125%; 5 days at 0.025%
Decoquinate 0.003% (Rhone-Poulenc)	Deccox	1970	0
Sulfadimethoxine, 0.0125% + ormetoprin, 0.0075% (Hoffmann-La Roche)	Rofenaid	1970	5
Monensin, 0.01-0.0121% (Elanco)	Coban	1971	0
Robenidine, 0.0033% (American Cyanamid)	Robenz, Cycostat	1972	5
Lasalocid, 0.0075-0.0125% (Hoffmann-La Roche)	Avatec	1976	3
Salinomycin, 0.004-0.0066% (Agri-Bio)	Bio-Cox	1983	0
Halofuginone, 3 ppm (Hoechst-Roussell Agri-Vet)	Stenorol	1987	5
Narasin, 54-72g/T (Elanco)	Monteban	1988	0
Madurimicin, 5-6 ppm (American Cyanamid)	Cygro	1989	5
Narasin + nicarbazin, 54-90 g/T (Elanco)	Maxiban	1989	5
Semduramycin, 25 ppm (Pfizer)	Aviax	1995	0

Source: (8)
FDA, Food and Drug Administration.

species of coccidia in chickens, several more in turkeys, and many others in other hosts. A drug may be efficacious against one or several of these parasites, very few drugs are equally efficacious against all.

Mode of Action. Each class of chemical compound is unique in the type of action exerted on the parasite, and even in the developmental stage of the parasite most affected. The chemical mode of action of some drugs is known to be a highly detailed event, while the action of other drugs remains a mystery. The sulfonamides and related drugs compete for the incorporation of PABA and metabolism of folic acid. Amprolium competes for absorption of thiamine by the parasite. The quinoline coccidiostats and clopidol inhibit energy metabolism in the cytochrome system of the coccidia. The polyether ionophores upset the osmotic balance of the protozoan cell by altering the permeability of cell membranes for alkaline metal cations.

The coccidia are prone to attack by drugs at various stages in development in the host. Totally unrelated drugs may attack the same stage of parasite. The quinolones and ionophores arrest or kill the sporozoite or early trophozoite. Nicarbazin, robenidine, and zoalene destroy the first- or second-generation schizont, and the sulfonamides act on the developing schizonts and also on the sexual stages. Diclazuril acts in early schizogony with *E. tenella*, but is delayed to later schizogony with *E. acervulina* and to the maturing macrogamete with *E. maxima*. The time of action in the life cycle has been construed as having significance in the use of

drugs in certain types of programs in which immunity is desired, but there is no good evidence that this is true under practical conditions.

Coccidiocidal vs. Coccidiostatic. Some drugs kill the parasite, but others only arrest development. When coccidiostatic medication is withdrawn, arrested parasites may continue to develop and contaminate the environment with oocysts. In such cases, a relapse of coccidiosis is possible. In general, the coccidiocidal drugs have been more successful than those that are coccidiostatic.

Effects of Drugs on the Chicken. Most compounds used in animal feeds have good "selective toxicity," providing toxicity for the parasite but being nontoxic to vertebrates. Unfortunately, toxicity and side effects of drugs on the host are possible where formulation errors lead to overdose. Sometimes a drug may exhibit side effects at the recommended use level. Some of the toxicity may be the result of management, genetics, nutrition, or other interaction, and in other cases the margin of safety is just too narrow. Environmental interaction is possible with nicarbazin, which interacts with high temperatures to produce excess mortality. Also nicarbazin is highly toxic to layers, first causing a bleaching of brown-shelled eggs, mottling of yolks, reduced hatchability, and reduced production. The ionophores are highly toxic at elevated doses, causing a transient paralysis in mild overdoses, or a permanent paralysis and mortality in more severe cases. Monensin was once thought to interact with methionine to reduce feather growth, but this relationship is not clear. Under some conditions, lasalocid will stimulate water consumption and excretion, resulting in a wet litter. With slight overdoses, most of the ionophores depress weight gain under laboratory conditions. A withdrawal period of 5–7 days is often practiced to allow "compensatory growth" to make up for the lost gain. The ionophores are known for their toxicity to other animals. Thus, monensin and salinomycin are highly toxic to horses. The lethal dose-50% (LD_{50}) for monensin in horses is about 2 mg/kg body weight. Salinomycin is highly toxic to turkeys at levels above 15 g/ton and causes excessive mortality at the level recommended for use in chickens (60 g/ton), while monensin and lasalocid are well tolerated in turkeys at the level used for chickens.

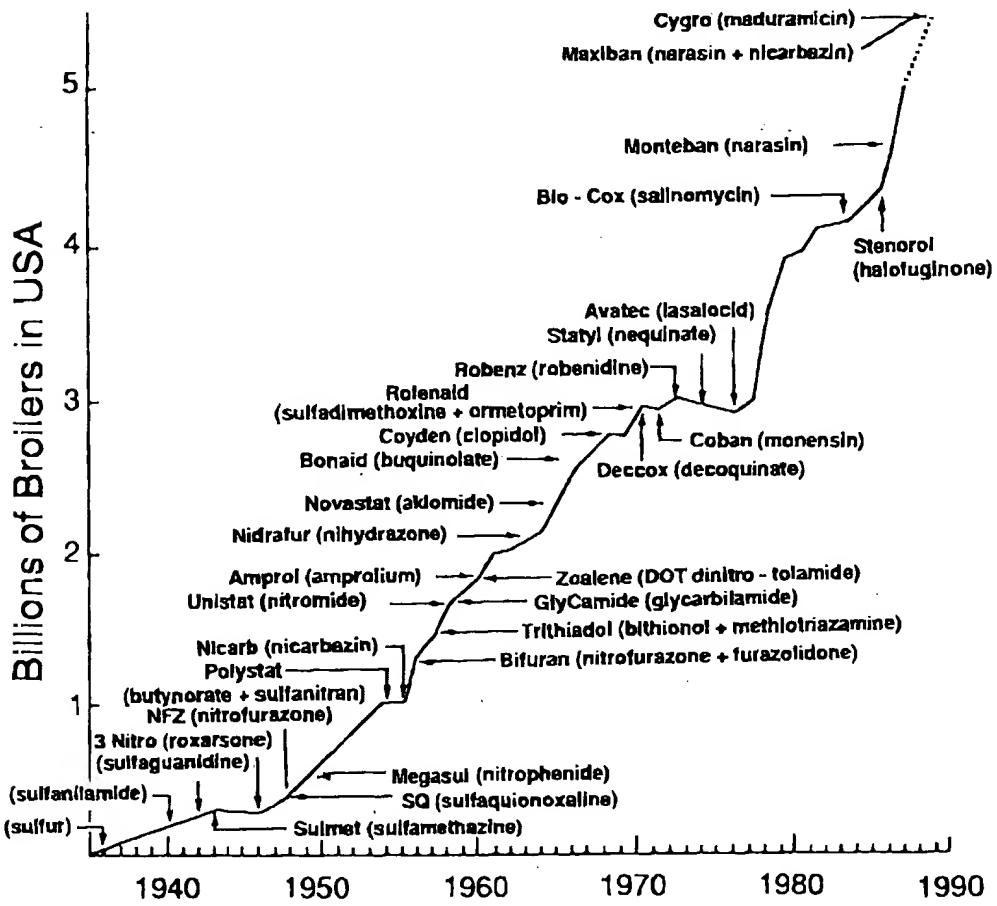
PROGRAMS FOR USE OF ANTICOCCIDIAL DRUGS IN BROILERS. In broilers, the objective is usually to produce the maximum growth and feed efficiency with minimum of disease, while in layers or breeders the objective may be immunization (Fig. 34.5).

Continuous Use of a Single Drug. Often, a single product will be used from day 1 to slaughter, or with a withdrawal period of 3–7 days. Most products are approved for use until slaughter, but producers withdraw medication for economic or other reasons.

Shuttle or Dual Programs. The use of one product in the starter and another in the grower feed is called a "shuttle" program in the United States and a "dual" program in other countries. The shuttle program is usually intended to improve coccidiosis control. Intensive use of the polyether ionophore drugs for many years produced strains of coccidia in the field that have "reduced sensitivity" to the ionophores. It is a common practice to use another drug such as nicarbazin or halofuginone in either the starter or grower feed to bolster the anticoccidial control and take some pressure off of the ionophore. The use of shuttle programs is thought to reduce buildup of drug resistance. In 1988, approximately 80% of the producers used some type of shuttle program.

Rotation of Products. It is considered sound management to make changes in anticoccidial drug use. Most producers consider changes in the spring and in the fall. Rotation of drugs may improve productivity because of the buildup of isolates or species of coccidia that have reduced sensitivity after products have been used for a long time. Producers often notice a boost in productivity for a few months after a change of anticoccidial drugs.

DRUG RESISTANCE. The development of tolerance of drugs by coccidia after exposure to medication is the most serious limitation to the effectiveness of products. Surveys reveal widespread drug resistance in coccidia in the United States, South America, and Europe (11, 14, 15, 17, 20, 23). Even though coccidia develop less resistance to some drugs than to others, long-term exposure to any drug will produce a loss in sensitivity and, eventually, resistance. Drug resistance is a genetic phenomenon, and once established in a line of coccidia, will remain for many years or until selection pressure and genetic drift forces return to sensitivity in the population. Drugs such as the quinolones and clopidol have a well-defined mode of action, and resistance develops quickly as coccidia are selected with cytochromes, which do not bind as readily to the drug. The polyether ionophores, in contrast, have a more complicated mode of action involving the mechanisms of active transport of alkaline metal cations across cell membranes, and it has taken many years for coccidia to become tolerant, and in some cases, completely resistant. Many other drugs appear to be intermediate in selecting resis-



34.5. Broiler production (USDA figures) and year of introduction of new anticoccidial drugs. Generic names begin with lower case letters and trade names begin with capital letters. (Avian Dis)

tance in coccidia. The primary defense against drug resistance is the use of less intensive programs, shuttle programs, and frequent rotation of drugs. Rotation of programs, used alone, will not prevent the development of resistance because the periods of use of drugs between changes is often adequate for resistance development.

ANTICOCCIDIAL DRUGS USED FOR BROILERS IN THE UNITED STATES. The products currently approved for use in chickens in the United States are listed in Table 34.3. Not all are still available commercially, but the approvals remain. Those used at present include monensin, salinomycin, and lasalocid (polyether ionophores), nicarbazin, amprolium + ethopabate, decoquinate, clopidol, sulfadimethoxine + ormetoprim and sulfaquinoxaline. Other products listed with approvals but lacking in significant activity include chlortetracycline, oxytetracycline, and nitrofurans. These products may prevent mortality from coccidiosis when given at high levels because of antibacterial activity, but are not of much value in general use. The polyether ionophores became the drugs of choice for prevention of coccidiosis in 1972, and remain the most extensively used today. Other drugs, such as nicarbazin and halofuginone, are used mostly in shuttle programs as an adjunct to the ionophores.

New drugs pending approval in the United States, which are used in Europe, South America, or Asia, include diclazuril and toltazuril (synthetic triazines).

DRUGS AND PROGRAMS USED IN BREEDERS AND LAYERS. Pullets started on the floor and later reared as caged layers do not need immunity to coccidiosis. They are often protected against coccidiosis with preventive medication, as with broilers until they are moved to cages. Breeder pullets that will be kept on the floor during lay should have an immunity to coccidiosis. Natural immunity after mild infection is usually achieved by one of two approaches. Controlled exposure can be given by means of commercial products (Coccivac, Immucox, or Paracox). The program calls for a light, harmless initial exposure, which must be reinforced by two to three repeated natural life cycles. Careful supervision of management is required to provide adequate immunity. The second approach relies upon a natural exposure assuming the presence of oocysts of important species. A broad-spectrum anticoccidial drug is fed to provide protection for 6–12 wk. Some producers reduce the level of the drug during the final 4 wk in a "step-down" program. Occasionally, oocyst numbers may be insufficient to provide adequate exposure to all species. Outbreaks of *E. necatrix* have sometimes occurred at 8–16 wk after all medication has been stopped. Climatic and seasonal conditions may add to the inherent uncertainties of this method. Neither program is foolproof, and damaging outbreaks may occur.

NEW VACCINES TO PREVENT COCCIDIOSIS. The considerable research on coccidiosis vaccines in recent years has produced interesting results and may eventually lead to a commercial product. Along conventional lines, Coccivac-style vaccines have been prepared from live but attenuated lines of oocysts. The success of these vaccines may depend more on a novel approach to administration rather than attenuation. One product is encapsulated in alginate beads, then mixed into the starter feed for "trickle administration." Another is be given by spraying the oocysts directly into feed or water in the poultry house.

Monoclonal antibody technology has led to identification of coccidial proteins protective against infection when inoculated into young chicks. These proteins can be made in quantity if the gene that encodes the protein is cloned into a bacterial cell. Research is in progress identifying broad-spectrum antigens and appropriate routes of administration.

DISINFECTION AND SANITATION. Older recommendations for coccidiosis control often suggest directions for sanitation and disinfection to prevent outbreaks. Most of these are no longer considered valid since: 1) there have been too many failures in such programs; 2) oocysts are extremely resistant to common disinfectants; 3) complete house sterilization is never complete; and 4) an oocyst-sterile environment for floor-maintained birds could prevent early establishment of immunity.

If birds are kept in self-cleaning cages, immunity is not essential, and outbreaks of coccidiosis occur only rarely, usually in single rows of cages in which there has been accidental fecal contamination of feed or water.

COCCIDIOSIS IN TURKEYS.

Coccidiosis in turkeys is common, but is often unrecognized because the lesions in turkeys are less spectacular than those in chickens. Several species infect turkeys, but only about four are economically important. Typical signs of coccidiosis in turkeys are watery or mucoid diarrhea, ruffled feathers, anorexia, and general signs of illness. Recovery is quick, so lesions could go undetected at necropsy. Several species have been found in commercial turkey farms throughout the United States (6). Coccidia infecting domestic turkeys also infect wild turkeys. Range-rearing of turkeys can add significantly to the exposure of wildlife to coccidiosis and other diseases.

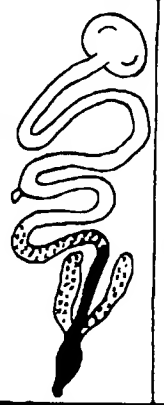
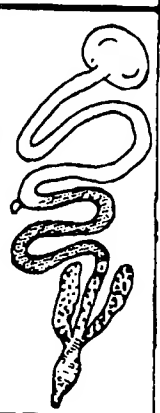
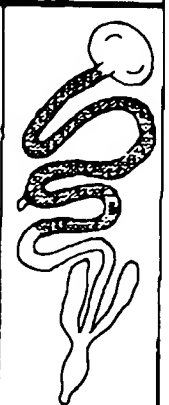
Turkeys of all ages are susceptible to primary infection, but birds older than 6–8 wk are considered more resistant to the disease; they can suffer weight loss and morbidity, but are not killed as easily as are younger birds. Reductions in rate of weight gain are often unrecognized until adequate coccidiosis control measures have been instituted.

Etiology. Seven species of *Eimeria* have been described in turkeys in the United States. Identifying characteristics of each species are listed in Table 34.4. *E. innocua and E. subrotunda* have been so rarely recovered that the validity of these species should be listed as doubtful.

Species described from the turkey include *Isospora* and *Cryptosporidium* (see next section) as well as *Eimeria*. The strictly intestinal *Eimeria* spp. contrast with *Cryptosporidium*, which may cause both respiratory and intestinal infection (13). The pathogenic species of *Eimeria* are *E. adenoeides, E. meleagrimitis, E. gallopavonis,* and *E. dispersa.* Differentiation of oocysts of the pathogenic species from those of milder species is difficult because some of the species are poorly described.

***EIMERIA ADENOEIDES* MOORE AND BROWN 1951.** Gross lesions appear primarily in the ceca, but extend to the lower small intestine and cloaca. Cecal contents are often hardened into a core consisting of mucosal debris. The cecal and/or intestinal wall is often swollen and edematous. Oocysts are ellipsoidal and have a high shape index (length/width = 1.54). The oocysts average 25.6 × 16.6 μm.

Table 34.4. Diagnostic characteristics of Eimeria in turkeys

SPECIES → CHARACTERISTICS	E. adenoeides	E. dispersa	E. gallopavonis	E. innocua	E. meleagridis	E. meleagrimitis	E. subrotunda
Lesions Occasional lesions Parasites no lesion Species distinctive							
Macroscopic lesions	liquid feces with mucus and flecks of blood, loose whitish cecal cores	cream-colored serosal surface, dilation of intestine, yellowish mucoid feces	edema, ulceration of mucosal ileum, yellow exudate, flecks of blood in feces	none	cream-colored ceca, formation of caseous plug, a few petechial hemorrhages	spotty congestion and petechiae from duodenum to ileum, dilation of jejunum, casts	none
Length × Width (in µm) Length = Width =	Av = 25.6 × 16.6 18.9 - 31.3 12.6 - 20.9	Av = 26.3 × 21.0 21.8 - 31.1 17.7 - 23.9	Av = 27.1 × 17.2 22.7 - 32.7 15.2 - 19.4	Av = 22.4 × 20.9 18.57 - 25.86 17.36 - 24.54	Av = 24.4 × 18.1 20.3 - 30.8 15.4 - 20.6	Av = 19.2 × 16.3 15.8 - 26.9 13.1 - 21.8	Av = 21.8 × 19.8 16.48 - 26.42 14.21 - 24.44
Oocyst shape and index length/width	ellipsoidal 1.54	broadly oval 1.24	ellipsoidal 1.52	subspherical 1.07	ellipsoidal 1.34	ovoid 1.17	subspherical 1.10
Minimum sporulation	24 hr	35 hr	15 hr	under 45 hr	24 hr	18 hr	48 hr
Prepatent period (minimum)	103 hr	120 hr	105 hr	114 hr	110 hr	103 hr	85 hr
Refractile body	yes	no	yes	no	yes	yes	no
Pathogenicity	+ + + +	+	+ + + +	none	none	+ + + +	none

NOTE: Characteristics compiled from original descriptions.

PATHOGENICITY. *E. adenoeides* is one of the most pathogenic of the turkey coccidia. Experimental infections of 25,000–100,000 oocysts in young poults may produce mortality up to 100% on the 5th or 6th day PI. Turkeys several months old may lose considerable weight after infection. Outward signs of infection are apparent after 4 days PI. Feces are frequently fluid, may be blood-tinged, and may contain mucous casts. White or gray caseous cores may be produced in the ceca. The lesions heal quickly, so no evidence of infection may be seen soon after the acute phase unless the cecal core remains.

GROSS LESIONS AND HISTOPATHOLOGY. By the 4th day PI, the intestine may suffer congestion, edema, petechial hemorrhage, and mucus secretion. Five days PI, the ceca contain white caseous material, which condenses into a core. The serosal surface of the intestine appears pale and may be edematous and dilated.

Invasion of the submucosa by heterophils occurs throughout the intestine, especially in the lower small intestine and ceca. Epithelial cells at the tips of villi are most often invaded, but deep glands may also be parasitized. Edema is common deep in the muscular layers as the infection progresses. After the 5th day, regeneration of lost mucosa is rapid.

***Eimeria dispersa* Tyzzer 1929.** The small intestine, principally the midgut region, is commonly parasitized, but some infection may occur in the cecal necks. Oocysts are large (average, 26.1 × 21.0 µm) and broadly ovoid (index = 1.24). Sporozoites lack a refractile body, and the oocyst wall is distinctively contoured and lacks the double wall common to other species. The prepatent period is 120 hr, longer than for other species.

PATHOGENICITY. Compared with some of the other species, the pathogenicity is low, but infection with 1,000,000–2,000,000 oocysts can cause reduction in rate of weight gain and diarrhea in young poults.

NATURAL AND EXPERIMENTAL HOSTS. The natural host of this species is apparently the bobwhite quail, in which the parasite is more pathogenic than in turkeys. This is the only *Eimeria* in chickens or turkeys known to infect more than one species. Experimental inoculation has produced patent infections in domestic and wild turkeys, Hungarian partridge (Perdix perdix), ruffed grouse (*Bonasa umbellus*), sharp-tailed grouse (*Pediocetes phasianellus campestris*), Japanese and bobwhite quail, and other pheasants. Infection in chickens often requires immunosuppression.

GROSS LESIONS AND HISTOPATHOLOGY. Three days PI, the duodenum appears cream colored on the serosal surface. Later, the entire intestine may become dilated with thickening of the wall. Dilation continues on the 5th and 6th days, along with secretion of a cream-colored mucoid material containing denuded epithelium from the duodenum. Individual villi may become so dilated as to be visible to the naked eye.

The duodenum shows edema and progressively increasing congestion of capillaries. Separation of the epithelium and basement membranes may result in the lamina propria being exposed to a fibrin network or an open fluid-filled space. Necrosis is common on distal tips of villi. Parasites do not invade the glands.

***Eimeria gallopavonis* Hawkins 1952.** Lesions are restricted to the area posterior to the yolk sac diverticulum and tend to be most severe in the lower small intestine and large intestine. Some foci of infection may be seen in the ceca. Oocysts are elongate, averaging 17.1 × 17.2 µm (index = 1.52).

PATHOGENICITY. Experimental infection with 50,000–100,000 oocysts causes mortality of 10–100% in 2- to 6-wk-old poults. Mortality occurs 5–6 days PI.

GROSS LESIONS AND HISTOPATHOLOGY. Marked inflammatory and edematous changes on the 5th to 6th day are followed by sloughing of soft white caseous necrotic material containing numerous oocysts and flecks of blood on the 7th and 8th days.

***Eimeria meleagridis* Tyzzer 1929.** Visible lesions may be seen in the ceca with yellow-white caseous cores, but this species is considered virtually nonpathogenic. Oocysts resemble those of other pathogenic species in the ceca, and differentiation is difficult.

PATHOGENICITY. Most studies have characterized this species as almost nonpathogenic. Two to five million oocysts produce little effect on growth of 4- to 8-wk-old poults. Earlier reports indicating greater pathogenicity may have come from mixed infections with *E. adenoeides*.

GROSS LESIONS AND HISTOPATHOLOGY. Nonadherent cream-colored caseous cecal cores are characteristics of infection in young poults. The core may be passed intact. The mucosa is somewhat thickened and may contain petechial hemorrhages in dilated portions of the ceca. The plugs disappear 5.5–6 days PI, and many oocysts may be found in cecal contents.

Edema and lymphocytic infiltration may be seen

histologically, but less extensively than with *E. adenoeides* and *E. gallopavonis*. First-generation schizonts develop in surface epithelium of the small intestine, but later stages occur in the cecal epithelium.

***EIMERIA MELEAGRIMITIS* TYZZER 1929.** Infection with E. meleagrimitis is primarily upper intestinal, but may spread throughout the small intestine in heavy infections. This is the most pathogenic of the upper-intestinal coccidia in turkeys. The oocysts are small (average, 19.2 × 16.3 µm) and ovoid.

PATHOGENICITY. Experimental infection of young poults produces morbidity and mortality, lost weight gain, dehydration, and general unthriftiness. Inoculation of 200,000 oocysts produces some mortality and morbidity, but this species is not as pathogenic as *E. adenoeides*.

GROSS LESIONS AND HISTOPATHOLOGY. Infected birds show signs of dehydration. In the duodenum, enlargement and congestion are marked on the 5th and 6th days of infection. Large amounts of mucus and fluid may be found in the lumen. Feces may contain occasional flecks of blood and mucous casts 5–7 days PI.

The tips of villi are most commonly parasitized, and the epithelium may be completely denuded, although hemorrhage is rare. Eosinophilic infiltration may begin as early as 2 hr PI and is extensive at the height of the infection.

UNDESCRIBED SPECIES. Several species of coccidia that do not fit descriptions of established species have been isolated from wild or domestic turkeys, but have not been adequately described or named. Thus, some difficulty may be expected in speciating coccidia found in field cases unless the pathology and appearance are distinctive.

Prevention and Control of Turkey Coccidiosis. Drugs effective in chickens are generally effective in turkeys, but the optimal level of application may vary and the toxicity of some drugs is significantly higher in turkeys than in chickens.

TREATMENT. As in chickens, treatment of outbreaks in turkeys is less desirable than prevention by chemotherapy or immunization. When treatment is necessary, application of amprolium (0.012–0.025% in water) or a sulfonamide (dosage depending on drug, often given 2 days on drug, 3 days off, and 2 days on, sometimes repeated a 2nd wk). The toxicity of sulfonamides limits their usefulness for turkeys.

CONTROL BY CHEMOTHERAPY. Most producers use anticoccidial drugs continuously in the feed at least 8 wk. Generally, poults are confined to a brooding facility at that time. Later, the birds may be moved to range or to other facilities. Drugs approved for use in feed include amprolium (0.0125–0.25%), butynorate (0.0275%), sulfaquinoxaline (0.0175%), sulfadimethoxine (0.006–0.25%) + ormetoprim (0.00375%), or monensin (54–90 g/ton), halofuginone (1.5–3.0 ppm), and lasalocid (75–125 ppm).

PREVENTION WITH PLANNED IMMUNIZATION. The principle of immunization by exposure to a small number of pathogenic oocysts of the important species of *Eimeria* was developed with chickens and is represented by a single product for turkeys in the United States (Coccivac-T, Sterwin, Millsboro, Delaware) and in Canada (Immucox, Vetech, Guelph, Ontario). The inoculum is sprayed on the feed during the first 1–7 days and causes a mild infection. There are risks inherent in use of virulent strains of coccidia, and occasional treatment at 3–4 wk of age is necessary if one of the species multiplies too rapidly, but the program has been used with success in most instances.

COCCIDIOSIS IN GEESE.

Numerous species of coccidia have been described from domestic and wild geese. The most prevalent and damaging in commercial flocks are *E. truncata*, which causes renal coccidiosis, and *E. anseris* which causes intestinal coccidiosis. Renal coccidiosis may produce high mortality from blockage of kidney function in young goslings. Coccidia may be introduced into domestic flocks by migrating and resident wild geese.

***EIMERIA TRUNCATA* RAILLET AND LUCET 1891.** Flock losses due to renal coccidiosis have been reported as high as 87% in Iowa. Geese aged 3–12 wk are affected, although the disease is most acute in goslings. Signs of infection include depression, weakness, diarrhea with whitish feces, and anorexia. Eyes become dull and sunken and wings are drooped. Survivors may show vertigo and torticollis. Birds quickly develop immunity to reinfection.

Oocysts and endogenous stages of *E. truncata* are found only in the kidneys or cloaca near the junction of the ureters. Diagnosis of *E. truncata* is assured by finding the distinctive oocysts in the kidneys and ureters. Oocysts average 21.3 × 16.7 µm and have truncated ends.

NATURAL AND EXPERIMENTAL HOSTS. Although thorough cross-infection experiments have not been done in most cases, *E. truncata* has been

reported from domestic and wild geese, ducks, and swans.

GROSS LESIONS AND HISTOPATHOLOGY. The kidneys may be enlarged and protrude from the sacral bed. The normal reddish brown is altered to light grayish yellow or grayish red. Pinhead-sized grayish white foci or hemorrhagic petechiae may be seen; they contain numerous oocysts and accumulations of urates. Invading and growing parasites may distort the kidney tubules to many times the normal size. Eosinophils and signs of necrosis are present in focal areas.

***EIMERIA ANSERIS* KOTLAN 1933.** The oocysts average 19.2 × 16.6 µm. Differentiation from the 14 species listed by Pellerdy (25) may be difficult.

PATHOGENICITY. *E. anseris* may produce anorexia, tottering gait, debility, diarrhea and morbidity, and sometimes mortality. The small intestine becomes enlarged and filled with thin reddish brown fluid. Catarrhal inflammatory lesions are most intense in middle and lower portions of the small intestine. There may be large whitish nodules or a fibrinous diphtheroid necrotic enteritis. Under dry pseudomembranous flakes, the oocysts and endogenous stages of the parasite are found in large numbers. Parasite stages invade epithelial cells of the posterior half of the intestine in closely packed rows. Developing gametocytes penetrate deeply into subepithelial tissues of the villi.

TREATMENT. Various sulfonamide drugs have been used in treatment of renal and intestinal coccidiosis of geese. Some studies indicated a favorable response, but, unfortunately, there have been no controlled experiments.

COCCIDIOSIS IN DUCKS.

Coccidiosis in ducks is sporadic but is of sufficient frequency to warrant more attention from researchers. Cases involving moderate to heavy mortality have been reported on domestic duck farms in New York, New Jersey, Hungary, and Japan. Coccidia were recovered from every farm sampled on Long Island, New York. Clinical and subclinical coccidiosis appears to be quite common, and can produce morbidity and mortality as well as poor performance.

SPECIES OF COCCIDIA AND DESCRIPTIONS. Although 13 species of coccidia have been reported from domestic and wild ducks, the descriptions are often insufficient to use in diagnosis (25). Many species will remain in doubt until further work is completed. Coccidia in ducks may be of Eimeria, Wenyonella, or Tyzzeria. The genus can readily be determined from the sporulated oocyst. The oocysts of Eimeria have four sporocysts, each containing two sporozoites; Wenyonella have four sporozoites, each with four sporozoites; and Tyzzeria have eight naked sporozoites not contained within sporocysts.

Tyzzeria perniciosa Allen 1936, from domestic ducks in the United States, have thin-walled oocysts measuring 10–12.3 × 9–10.8 µm and sporulate to produce eight free sporozoites.

Wenyonella philiplevinei Leibovitz 1968 is the best described of the coccidia from ducks. It is found in the lower intestine from the posterior jejunal annular band to the cloaca. The prepatent period is 93 hr. The oocysts have three-layered walls, measure 15.5–21 × 12.5–16 µm (average, 18.7 × 14.4), have a micropyle at one end, 1–2 polar granules, and no oocyst residuum. Sporulation results in four sporocysts/oocyst, each containing four sporozoites.

PATHOGENICITY OF DUCK COCCIDIOSIS. Signs of infection with *T. perniciosa* usually include anorexia, weight loss, weakness, distress, morbidity, and up to 70% mortality. Hemorrhagic areas are common in the anterior portion of the intestine but may be found throughout. Bloody or cheesy exudate is common. The epithelial lining may be sloughed in long sheets. Parasite invasion may extend through the mucosal and submucosal layers as deep as the muscular layers. Acute hemorrhage as early as the 4th day may be followed by death on the 5th to 6th day.

With *W. philiplevinei*, the effects are limited to 72–96 hrs PI. Occasional petechial hemorrhages appear in the posterior ileal mucosa. Diffuse congestion is found in lower intestinal mucosa. In severe infections, mortality may occur on the 4th day.

COCCIDIOSIS IN PIGEONS.

Coccidiosis in pigeons is similar to, but less severe than, that caused in chickens by *E. necatrix*. Young pigeons suffer the greatest losses, but mortality may occur in birds as old as 3–4 mo.

The most frequently occurring species of coccidia in pigeons is *E. labbeana* (Labbe 1896) Pinto 1928. Oocysts are spherical or subspherical, averaging 19.1 × 17.4 µm.

PATHOGENICITY. Mortality of 15–70% has been reported in young pigeons in various parts of the world. Subclinical infections may persist in older birds for long periods. Immunity does not appear to be as "self-limiting" as reported for other species. Common signs of infection are anorexia, greenish diarrhea, marked dehydration, and emaciation. Droppings may be blood tinged, and the entire digestive tract may be inflamed. The common condition of "going light" is frequently attributed to coccidiosis.

TREATMENT. Favorable response has been reported after use of sulfonamides in drinking water at the same or half the level recommended for chickens. A product was introduced in 1987 in France and Belgium for specific use in pigeons. The active ingredient is Clazuril, a close relative of the Diclazuril under development for use in chickens. This product is highly effective in treating coccidiosis in pigeons.

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CRYPTOSPORIDIOSIS

William L. Current

INTRODUCTION. Cryptosporidiosis is caused by small coccidian parasites of the genus *Cryptosporidium* that live within the microvillous region of epithelial cells of the respiratory and gastrointestinal tracts of vertebrates. Naturally occurring infections have been reported from at least nine different avian hosts. In chickens, turkeys, and quail, these parasites are primary pathogens that can produce respiratory and/or intestinal disease, resulting in morbidity and mortality. Reviews of the biology of *Cryptosporidium* spp. are now available (5, 11, 12, 30).

HISTORY AND TAXONOMY. Clarke (2) in 1895, observed what may have been a species of *Cryptosporidium* in mice. The type species *C. muris*